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S. aureus Genotyping Kit 2.0

Array Hybridisation Kit for DNA-based detection of resistance genes and pathogenicity markers of *Staphylococcus aureus* and assignment of unknown *S. aureus* isolates to known strains

For Research Use Only. Not for Use in Diagnostic Procedures.

CONTENT

BACKGROUND

The CLONDIAG *S. aureus* Genotyping Kit 2.0 allows DNA-based detection of resistance genes and pathogenicity markers of *Staphylococcus aureus* and assignment of unknown *S. aureus* isolates to known strains.

RNA-free, unfragmented genomic DNA from pure and monoclonal *S. aureus* colony material is amplified approximately 40-fold and internally labelled with biotin-dUTP using a linear amplification protocol. In contrast to standard PCR, only one antisense primer per target is used resulting in single stranded (ss) DNA reaction products. This allows a simultaneous sequence specific labelling and amplification of an essentially unlimited number of targets. However, sensitivity is lower than in a standard PCR (whereas contamination with undesired amplicons is nearly impossible) and for that reason the method is restricted to colony material and cannot be performed on samples such as swabs or pus. The resulting biotin-labelled ssDNA is transferred and hybridised to DNA oligonucleotide microarrays with 336 probes for different genetic markers and a biotin staining control. Most of them are printed in two duplicate spots.

The target set consists of a variety of species markers, virulence-associated genes including genes encoding exotoxins, antibiotic resistance genes, genes encoding microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), various enzymes and other types of markers [1] .

Spot recognition is performed automatically based on digital images of the arrays. The overall pattern is analysed automatically for the presence or absence of specific genes and it is compared to a database of strain profiles allowing assignment to clonal complexes and strains [1, 2].

In the recent (2.0) version of the assay, additional probes for the newly described methicillin resistance gene *mecC* [3, 4] and another marker specific for SCC*mec* XI (*blaZ*-SCC*mec* XI) were introduced.

GENERAL INSTRUCTIONS FOR USE

Intended Use

For Research Use Only. Not Intended for Use in Clinical Diagnostics.

This kit allows genotypic characterisation of bacterial cultures from *S. aureus* isolates for research and epidemiological applications. It must not be used as a substitute for phenotypic susceptibility tests and for the guidance of antibiotic therapy. It cannot be used for other bacteria than *S. aureus*.

Specifications

Upon receipt, the assay components need to be stored at different temperatures as specified on the package insert. The assay is to be performed at an ambient temperature of 18-28 °C.

Technical Support

If you require any further information on this product please contact:

email: cct.home@clondiag.com

phone: +49 (0) 3641 3111 0

For up-to-date information regarding the kit, please visit our website at

http://www.alere-technologies.com

Safety Precautions

The kit is intended for use by personnel that are trained in microbiological and molecular methods. Preparation of DNA from pure S. aureus colonies (clones) requires expertise in microbiology, and the local regulations for handling of pathogenic microorganisms (biosafety level 2) are to be obeyed.

Isolated, cell-free S. aureus DNA may be processed without further biosafety precautions, although contamination with S. aureus or other bacteria needs to be ruled out.

Always wear protective clothes as required for laboratory work by your local regulations.

Material Safety Data Sheets (MSDS)

According to OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC: 1005, 1008(1999)] and the latest amendments to the European Union Directives 67/548/EC and 1999/45/EC, the enclosed reagents do not require a Material Safety Data Sheet (MSDS). They do not contain more than 1% of a component classified as hazardous and do not contain more than 0.1% of a component classified as carcinogenic. MSDS therefore are not provided. Nevertheless, the buffers may cause irritation if they come into contact with eyes or skin, and may cause harm if swallowed. The regular precautions associated with laboratory work should be obeyed (e.g. wear protective goggles, gloves and lab coat and avoid contact with the reagents). In case, any liquids are spilled, clean with disinfectant and/or laboratory detergent and water.

Alere assumes no liability for damage resulting from handling or contact with these products. If you have any questions please contact our Technical Support (see above).

Shipping Precautions

RID/ADR: *Kein Gefahrgut* / No dangerous goods

IMDG: No dangerous goods

IATA: No dangerous goods

REAGENTS AND DEVICES

Kit Components, Storage and Stability

All reagents are provided in a certain surplus amount (see below). In case of need, all components may also be ordered separately; please refer to the order numbers at the end of this manual. For pricing please contact your local representative or our customer service, respectively.

The expiry date can be found on each bottle and on the outer package. All components were been tested for stability for short term shipment (< 1 week) at ambient temperature (< 37 °C). The kit components with a rather limited stability are D1 and C3. All other components proved to be stable even six months after passing the kit expiry date.

Cell Lysis

- A1: Lysis Buffer
  Store at 18-28 °C (ambient temperature). Surplus: 50 %.
- A2: Lysis Enhancer (lyophilised)
  Store at 18-28 °C (ambient temperature). Centrifuge A2 tubes briefly prior to opening. Add 200 µL Buffer A1 to Lysis Enhancer before use. Mix well and store for less than 1 week at 2-8 °C. Sufficient for 96 isolations.

DNA Labelling and Amplification

- $B1^{ST2}$: Labelling Buffer/Master Mix
  Store at 2-8 °C. Surplus: 25 %.

Do not use $B1^{ST}$ Labelling Buffer/Master Mix from earlier kit versions for S. aureus Genotyping Kit 2.0!

- B2: Labelling Enzyme
  Store at 2-8 °C. Surplus: 50 %.

Hybridisation and Detection

- ArrayStrips (12 x 8 samples)

  Protected against light and sealed under inert gas. Store at 18-28 °C. After opening to be used within two weeks. Close the unused wells with caps, protect them against humidity and dust, and store them at a dark place. *Avoid any touching or scratching of the microarray surface at the bottom of the well. Do not store or handle unused wells at more than 60 % relative humidity since this may irreversibly corrode the spots.*

- StripCaps (24 units)

- C1: Hybridisation Buffer

  Store at 18-28 °C, protect against direct sunlight. Surplus: 100 %.

- C2: Washing Buffer 1

  Store at 18-28 °C, protect against direct sunlight. Surplus: 200 %.

- C3: HRP Conjugate 100 x

  Store at 2-8 °C, protect against direct sunlight. Surplus: 100 %.

- C4: Conjugate Buffer

  Store at 18-28 °C, protect against direct sunlight. Surplus: 200 %.

- C5: Washing Buffer 2

  Store at 18-28 °C, protect against direct sunlight. Surplus: 200 %.

- D1: Horseradish Peroxidase Substrate

  Store at 2-8 °C, protect against direct sunlight. Surplus: 50 %.

- CM: Reference DNA from *S. aureus* strain N315

  (GenBank accession number BA000018), 0.1 µg/µL. Store at 2-8 °C. Sufficient for 5-6 tests.

Instrumentation and Software

- ArrayMate Reader (to be ordered separately, for details see below)

  The *S. aureus* Genotyping Kit 2.0 may be used on the ArrayMate Reader only. The older

devices ATR01/03 are not suitable for reading ArrayStrip based assays. In case of any questions please contact your local distributor and/or Alere Technologies Jena.

- Iconoclust software (provided with the reader)
- Test specific software plug-in that contains information such as spot names, marker names, positions of the spots on the array. This plug-in is delivered with the reader. or can be downloaded from our website. Test-specific plug-ins will occasionally be updated. Please check the NEWS section of our website http://www.clondiag.com. Support is available via cct.home@clondiag.com or +49 (0) 3641 3111 0.

Components Required but not Provided

- Growth media for the cultivation of *S. aureus.* The test should be performed with colonies harvested from Columbia Blood Agar. Other media that contain blood may also suffice, but have not systematically been tested. Media that do not contain blood (Mueller-Hinton, or MRSA selective media) usually yield lower DNA concentration, they are not recommended. As well, liquid media should not be used because contamination or culture mixing cannot easily be ruled out.
- Equipment and consumables needed for the cultivation of *S. aureus* (incubator, inoculation loops, Petri dishes)
- Clumping factor/coagulase assays for confirmation of *S. aureus*
- DNA preparation kit: The assay was tested with the DNeasy Blood & Tissue Kit from Qiagen (cat# 69504), QIAamp Minikit (cat# 51306) and a DNA preparation kit for Qiagen´s EZ1 automated device (DNA Tissue Kit, cat# 953034).
  Please note: DNA isolation from S. aureus requires a pre-treatment with the Cell Lysis components A1/A2 (see below).
- Equipment needed for DNA isolation, e.g. pipettes, centrifuge, thermoshaker or automated device (see above)
- Photometer for measuring the concentration of DNA

- Equipment for DNA gel electrophoresis for quality control of DNA
- Thermocycler
- Thermoshaker

  We strongly recommend the BioShake iQ by Quantifoil Instruments (http://www.qinstruments.com/) equipped with a customised heating block designed to fit ArrayStrips. Alternatively, you may use Eppendorf's Thermomixer Comfort, equipped with a heating block for microtitre plates.
- Pipettes: suitable for volumes of 1 µL-5 µL, 90 µL, 100 µL, 200 µL, 1000 µL
- Multichannel Pipettes for 100-200 µL
- Reagent tubes suitable for PCR
- Ultrapure (PCR-grade) water

PROTOCOL

Culturing and Harvesting Bacterial Cells

S. aureus is a potential pathogen. All procedures for cultivation of the bacterium and DNA preparation need to be performed by properly trained staff in a biosafety level 2 facility.

Grow *S. aureus* on Colombia blood agar (overnight at 37 °C or 48 hrs at room temperature). Obtain confirmation of the identification as *S. aureus* (by katalase + coagulase/clumping factor assays or by other means) and make sure that you have a pure, monoclonal culture of *S.aureus*. Contamination with other bacteria, especially with other staphylococci, needs to be strictly avoided as they might carry the same resistance genes as certain *S. aureus* strains and thus can introduce false positive signals and patterns.

- Centrifuge A2 tube briefly, open it, add 0.2 mL of Lysis Buffer A1 to Lysis Enhancer A2 and dissolve.
- Add an inoculating loop full of monoclonal colony material of the *S. aureus* isolate into this A1/A2 reagent, vortex.

Loop empty Loop full

It is important to harvest enough bacteria; this is prerequisite for extraction of a sufficient amount of DNA.

Take an inoculating loop 1 mm in diameter filled with bacteria as shown in the righthand picture.

Extraction of DNA

The required sample type for the S. aureus Genotyping assay is 0.5-2 µg of intact genomic DNA from a single clone of S. aureus.

This is much more DNA than for standard PCR applications (see Introduction).

The DNA specimen needs to be free of RNA and it should not be fragmented.

This can be determined by agarose gel electrophoresis. DNA should not be prepared by disrupting *S. aureus* cells using bead beaters, ultrasonication or aggressive chemicals such as in alkaline lysis protocols. Most performance problems with the *S. aureus* Genotyping Kit 2.0 are due to insufficient amounts or quality of DNA preparation. We therefore strongly recommend to obey the protocols outlined below.

We also recommend performing an experiment with Reference DNA from *S. aureus* strain N315 (CM reagent) when establishing the procedure in your lab. This will help to detect the cause of possible problems.

Extraction of DNA by Spin Columns

- Incubate the colony material of the *S. aureus* isolate in A1/A2 for 30-60 min at 37 °C and 550 rpm in the thermoshaker.
- Proceed with the DNA preparation protocol of the DNA preparation kit. For the Qiagen DNeasy Blood & Tissue Kit that is as follows:
- Add 25 µL proteinase K (Qiagen Kit, or equivalent) and add 200 µL buffer AL (Qiagen Kit)
- Vortex briefly or shake vigorously.
- Incubate for 30-60 min at 56 °C and 550 rpm in the thermoshaker.
- Add 200 µL ethanol (96-100 %).
- Vortex the sample and centrifuge briefly.
- Transfer the complete content of the tube (including any precipitate) into a spin column that is placed in a 2 mL collection tube.
- Centrifuge at room temperature, time and speed need to be determined depending on viscosity of sample and type of centrifuge used. All liquid should be collected in the collection tube afterwards.
- Discard collection tube with liquids.

- Place the spin column in a new 2 mL collection tube (provided with the kit).
- Add 500 µL Buffer AW1.
- Centrifuge at room temperature.
- Discard collection tube with liquids.
- Place the spin column in a new 2 mL collection tube (provided with the kit).
- Add 500 µL Buffer AW2.
- Centrifuge at room temperature; the membrane of the spin column should be dry, and all liquid should be in the collection tube.
- Discard collection tube with liquids.
- Place the spin column in a clean 1.5 mL tube (provided with the kit).
- Add 50 µL Buffer AE (or PCR grade distilled water) directly onto the membrane of the spin column.
- Incubate at room temperature for 5 min to elute DNA.
- Centrifuge.
- Optional: add another 50 µL Buffer AE (or PCR grade distilled water) directly onto the membrane; incubate at room temperature for 1 min and centrifuge again.
- Discard the spin column.

Ethanol from Washing Buffers strongly inhibits the enzymes used in the assay.

A contamination with Washing Buffer might occur during elution of prepared DNA by droplets adhered to the funnel of the spin column. Thus, these funnels should be touched gently and dried with sterile filter paper or wipes prior to the elution step. Alternatively, prepared DNA can be heated briefly to evaporate ethanol (e.g. 10 min at 70 °).

- Check for DNA integrity and absence of RNA (e.g. by agarose gel). If necessary, you might perform another digestion step with additional RNAse (not provided). Measure DNA concentration (A_{260} method), it should not be less than 0.1 µg/µL. The concentration might be increased by heating and evaporation of water, or by using a speed vac centrifuge.

Extraction of DNA by Automated Device

The assay was tested with Qiagen´s EZ1. Other systems also can be used as well. However, performance should be checked with some known reference strains prior to routine use. Incubate the colony material of the *S. aureus* isolate in A1/A2 for 30-60 min at 37 °C and 550 rpm in the thermoshaker as described above (depending on the input sample volume required by the device you actually use, the A1/A2 mixture might be divided into two aliquots, and used for DNA preparation of two samples).

- Add 10 µL proteinase K and add 100 µL buffer AL.
- Vortex briefly or shake vigorously.
- Incubate sample 45-60 min at 56 °C and 550 rpm in the thermomixer.
- When the cells are lysed, proceed by performing the tissue lysis protocol (Bacteriacard) for Qiagen´s EZ1
- *For Qiagen´s EZ1*: Front row: empty elution tubes (1.5 mL); second row: tip holder with tips; third row: empty; back row: sample tube with conical tip (2 mL) containing the 200 µL sample volume. Set tissue lysis protocol with a set sample volume of 200 µL and an elution volume of 50 µL.
- Concentrate DNA and evaporate traces of solvents by heating the sample at 70 °C for 5-10 min.

Linear Amplification and Internal Biotin-Labelling

Please keep in mind the limited surplus of reagents whilst pipetting. The surplus of $B1^{ST2}$ labelling reagent is 25 %.

- Prepare a Master Mix by combining 4.9 µL of $B1^{ST2}$ labelling reagent (version 2.0, *Do not use $B1^{ST}$ from earlier kit versions!*) and 0.1 µL of B2 (Labelling Enzyme) per sample.
- Add 5 µL DNA (0.5-2 µg) prepared as described above to a 5 µL aliquot of the Master Mix. Do not forget to label the vial!

- Perform amplification in a pre-programmed thermocycler (such as Mastercycler gradient with heated lid, VWR, cat# 460-0108) according to the following protocol:

Pre-heat cover/lid to 105 °C	
300 sec at 96 °C	
50 (to 55) cycles with:	60 sec at 96 °C
	20 sec at 50 °C
	40 sec at 72 °C
Cool down to 4 °C, hold	

Please note: When using a different device, some adaptations, such as an increase of the number of cycles, might be necessary. Before establishing routine use, please test the protocol with a few known reference strains and the control DNA (CM) supplied with the kit.

Hybridisation

General Remarks - Handling of Arrays

Never touch the array surface!

Avoid complete drying of the array surface during processing!

Do not allow it to stay without liquid for more than two minutes!

Never rinse the wells with distilled water after hybridisation!

Unused wells should be capped during the whole procedure. The strips may be processed up to three times without a loss of quality of properly capped unused arrays. Close all wells that will not be used with a cap until you use these wells (for storage conditions after use: see section “Kit Components, Storage and Stability/Hybridisation and Detection”).

Always label your array strips with a laboratory marker at the recommended position. Never label them on the bottom or across the Data Matrix code! This may cause errors.

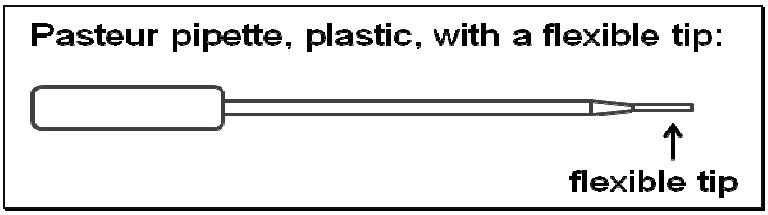
Avoid contact of Data Matrix code with organic solvents! The ArrayMate Reader needs the information encoded in the data matrix to perform the assay.

Avoid touching the bottom of the ArrayStrip and keep it clean.

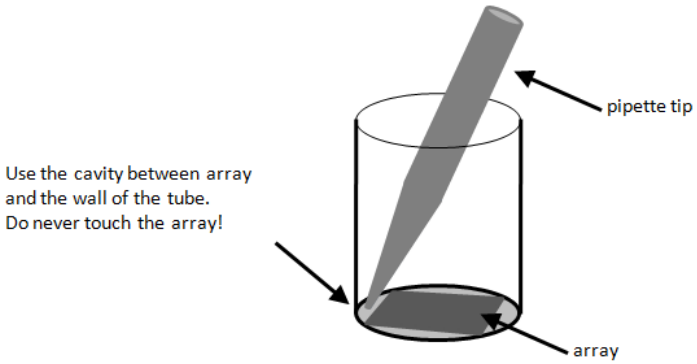
General Remarks - Handling of Liquids

We recommend the use of a multichannel pipette and reagent reservoirs. Please keep in mind the limited surplus of C1 (100 %).

We strongly recommend to remove the liquid by pipetting rather than by inverting the strips and flicking the liquids out. Fine tipped soft, disposable Pasteur pipettes are suited best (such as VWR cat# 612-2856).



Always place the pipette tip in the cavity between the array and the wall of the reagent well. If you touch the array surface, probes may be scratched off and this may cause an error.



General Remarks – the Substrate (Precipitating Dye) D1

It is recommended to fill an appropriate amount of substrate (precipitating dye D1) into a reaction tube and taken out of the refrigerator when starting the procedure to acclimatise it to room temperature/25 °C. Cold D1 may yield weak signals. D1 should be centrifuged briefly prior to use to remove bubbles as well as possible precipitates.

Triggered by peroxidase, the dye precipitates in case of positive reactions, but it is not covalently bound. The precipitate can be dissolved by vigorous shaking. Thus, the arrays must not be shaken, dropped or moved abruptly during the staining procedure and afterwards.

After completion of staining, remove and discard reagent D1 as completely as possible and scan immediately (ArrayMate). The dye precipitate fades slowly in presence of liquids.

General Remarks - Thermoshakers

The correct temperature within the vessels is essential; therefore always use the appropriate equipment for heating. Because of a possibly inhomogeneous distribution of the temperature within the heating block, and because of possible differences between displayed and actual temperatures, the use of different brands of thermoshakers might affect test performance. We tested the assay with BioShake iQ by Quantifoil Instruments (http://www.qinstruments.com/) equipped with a customised heating block designed to fit ArrayStrips, as well as with Eppendorf's Thermomixer Comfort equipped with a heating block for microtitre plates. Thus we

recommend the use of either device. Accordingly, two slightly different protocols are discussed here.

When using other devices, some modifications to the protocol might be necessary. Before establishing routine use, please test the protocol with a few known reference strains or the control DNA (CM) supplied with the kit.

Protocol for Quantifoil's BioShake iQ

- Switch on the thermoshaker and pre-heat it to 50 °C.
- Remove the amount of ArrayStrip(s) needed from the pouch.
- Insert the ArrayStrip(s) into the white frame. Assure the correct orientation (Data Matrix code close to row A) and proper fit.
- Pre-wash the array in two steps:
- First, PCR-grade distilled water, 200 µL per well at 50 °C, 5 min and 550 rpm.
- Second, C1 Hybridisation Buffer, 200 µL per well at 50 °C, 5 min and 550 rpm.
- Add 90 µL of buffer C1 to each tube with 10 µL labelled amplification product, mix gently.
- Remove the buffer from the well with the array and add the mixture of C1 and labelled amplification product.
- Incubate at 50 °C, 60 min and 550 rpm.
- Remove liquid and wash with 200 µL C2 Washing Buffer, pipette up and down four times, remove and discard.
- Add another 200 µL C2 Washing Buffer. Incubate at 30 °C, 10 min and 550 rpm.
- Meanwhile, prepare conjugate: For each experiment add 1 µL conjugate 100 x HRP to 99 µL C4 Conjugation Buffer. This mixture is stable for one day at room temperature; C3 is delivered with a surplus of 100 %, C4 is delivered with a surplus of 200 %.

Suggested pipetting scheme:

	1 well	2-3 wells	4-6 wells	7-10 wells	11-15 wells	16-20 wells	21-30 wells	31-40 wells
C3	1.5 µL	3.5 µL	7 µL	11 µL	16 µL	21 µL	32 µL	42 µL
C4	148.5 µL	346.5 µL	693 µL	1089 µL	1584 µL	2079 µL	3068 µL	4058 µL

- Remove and discard the Washing Buffer, and add 100 µL diluted conjugate to each well, incubate at 30 °C, 10 min and 550 rpm.
- Remove liquid and wash with 200 µL C5 Washing Buffer, pipette up and down four times, remove and discard.
- Add another 200 µL C5 Washing Buffer. Incubate at 30 °C, 2 min and 550 rpm.
- Remove and discard Washing Buffer, add 100 µL of D1 substrate (precipitating dye, at 25 °C, see above) per well.
- Incubate at 25 °C, 6 min ***but do not shake!***
- Remove liquid completely.
- The outside of the bottom of the ArrayStrips may cautiously be cleaned with wipes, gently pipetting up and down the D1.
- Scan and process (ArrayMate, see below).

Adapted Protocol for Eppendorf's Thermomixer Comfort

- Switch on the thermoshaker and pre-heat to 55 °C.
- Remove the amount of ArrayStrip(s) needed from the pouch.
- Insert the ArrayStrip(s) into the white frame. Assure the correct orientation (Data Matrix code close to row A) and proper fit.
- Pre-wash the array in two steps:
- First, PCR-grade distilled water, 200 µL per well at 55 °C, 5 min and 550 rpm
- Second, C1 Hybridisation Buffer, 200 µL per well at 55 °C, 5 min and 550 rpm

- Add 90 µL of Buffer C1 to each tube with 10 µL of labelled amplification product, mix gently.
- Remove the Washing Buffer from the well with the array and add the mixture of C1 and labelled amplification product.
- Incubate at 55 °C, 60 min and 550 rpm.
- Remove liquid and wash with 200 µL C2 Washing Buffer, pipette up and down four times, remove and discard.
- Add another 200 µL C2 Washing Buffer. Incubate at 30 °C, 5 min and 550 rpm.
- Meanwhile, prepare conjugate: For each experiment add 1 µL conjugate 100 x HRP to 99 µL C4 Conjugation Buffer. This mixture is stable for one day at room temperature; C3 is delivered with a surplus of 100 %, C4 is delivered with a surplus of 200 %.

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C3	1.5 µL	3.5 µL	7 µL	11 µL	16 µL	21 µL	32 µL	42 µL
C4	148.5 µL	346.5 µL	693 µL	1089 µL	1584 µL	2079 µL	3068 µL	4058 µL

- Remove and discard Washing Buffer, and add 100 µL diluted conjugate to each well, incubate at 30 °C, 15 min and 550 rpm.
- Remove liquid and wash with 200 µL C5 Washing Buffer, pipette up and down four times, remove and discard.
- Add another 200 µL C5 Washing Buffer. Incubate at 30 °C, 2 min and 550 rpm.
- Remove and discard Washing Buffer, add 100 µL of D1 substrate (precipitating dye, at 25 °C, see above) per well.
- Incubate at 25 °C, 6 min ***but do not shake!***
- Remove liquid completely.
- The outside bottom of the ArrayStrips may cautiously be cleaned with wipes, gently pipetting up and down the D1.

- Scan and process (ArrayMate, see below).

Data Analysis

Starting the ArrayMate Reader

We recommend to start the ArrayMate Reader after having started the hybridisation; this allows you to conveniently start the device and to import the worklist file (see below).

Please note that this is a short instruction only. For more detailed information please refer to the ArrayMate User Manual.

- Switch on the ArrayMate (1 st: main switch on the rear below the electric cable plug, 2 nd: operating switch on the bottom/left corner of the front side).
- Switch on the screen (switch righthand below the screen).
- Log on as **R&D User** (Research and Development User) for full access to test specific software (a default password will be provided together with the ArrayMate device).
  If you log on as **User**, you will obtain raw values only, but no identification as positives/negatives and no strain assignment. **Administrator** log on will allow manipulation of file folders and software; this should be done only upon direct advice of Alere`s IT team.
- The user interface will be loaded, ArrayMate performs internal testing. It requires slightly less than a minute.
- Click on the icon **New Run** (left upper edge of the screen). A suggestion for a run name / folder name for the new run appears in the top line of the screen. You may modify or change the experiment name at your convenience.
- Type in your operator ID **(optional)**.
- You may enter a comment into the **memo** field **(optional)**.

Worklist

A “Worklist” file allows to link an identifier such as a laboratory/sample number to a position of an array within the ArrayStrip. Please respect the rules of confidentiality and data protection. Worklists can be generated using spreadsheet software such as EXCEL (see below) but must be saved in the *.txt file format that can be imported into the test specific ArrayMate software. Do not use special characters (such as: ; ()[] / \ etc.).

- Create a list with at least three columns with obligatory headers in the following order: position / sample ID / assay ID (Table 1).
- Positions are continuously numbered from 1 to a maximum of 96. Position 1 would correspond to A1, 8 to H1, 9 to A2 and 96 to H12 (Table 2). Do not leave empty lines in the worklist. If you use EXCEL, position numbers should be typed into column A.
- Sample ID is strain / sample / laboratory numbers such as exported from your LIMS (or assigned in any different way). Patient name should not be used as Sample ID.
- The Assay ID enables the system to identify the current test and to correctly use information on layout, spot number, and identity etc.. *S. aureus* Genotyping Kit 2.0 has the Assay ID: 10620 (whereas the previous version of the *S. aureus* Genotyping Kit has Assay ID: 10248). *Assay ID must not be confused as this could lead to errors or loss of data.*
- You may add further columns and headers with notes and comments at your convenience. Information from these columns will not appear on the result screen or in the Test Report.
- We recommend using a printout of the worklist as template for pipetting.
- Safe the worklist as tab separated *.txt file on the memory stick provided together with the ArrayMate.
- To avoid confusion, make sure that worklists are named unambiguously or that worklists from earlier experiments are deleted.

Table 1: Example worklist:

Position	sampleID	assayID	comment
1	2013-12345	10620	
2	2013-12346	10620	
3	2013-12347	10620	
4	2013-12348	10620	
5	2013-12349	10620	
6	2013-12350	10620	
7	987654	10620	*Isolate referred from Dr. J. Doe.*
8	N315	10620	*Control strain*

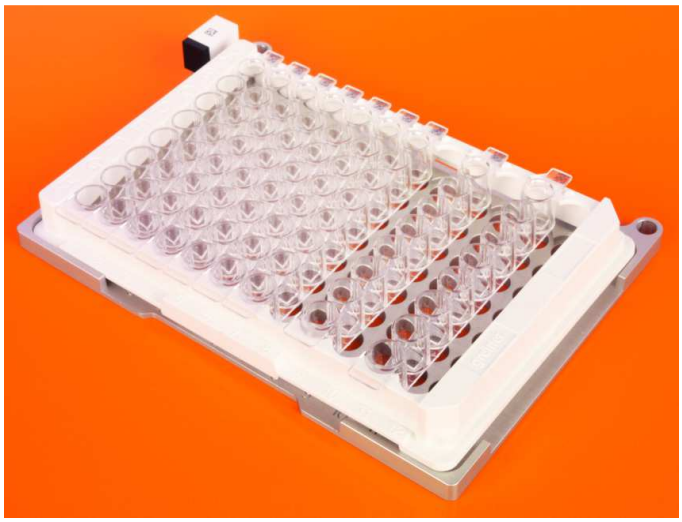
Table 2: Positions in the 96 well format

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
H	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

Data Acquisition in the ArrayMate Reader

- Insert your memory stick containing the worklist into any of the USB ports down to the right side of the ArrayMate.
- Press the button:  ; a folder selection dialog will open.
- Select your worklist (path: “My Computer/Removable Disk”).
- Open your selected worklist with **Enter** or the button **Open**.
- Press the button:  (your imported worklist opens in a separate window). Proofread. If the new window is empty, or if it was the wrong worklist, repeat the import.
- Press the button **OK**; the worklist window will close.

- Leave the memory stick attached to the ArrayMate if you intend to export *S. aureus* Genotyping Test Reports afterwards.
- Press the button **Next** (bottom / right on the screen; reader opens).
- Carefully insert the appropriate metallic adapter / frame into the ArrayMate. Do not apply any strong force. Assure proper fit, otherwise the images may be out of focus.
- Carefully insert the white frame with the array strips into the metallic adapter. Assure the correct orientation (Position A1 in the frame next to the Data Matrix code on the adapter) and proper fit, otherwise the images may be out of focus.



ArrayStrip frame with Strips inserted in accordance with the worklist.

Please note: ArrayStrips must be clean. They should not contain any liquids during analysis. Data Matrix codes must be clean. There must be no ArrayStripCaps on the wells that are to be analysed (however, unused wells should remain covered).

- Press the button **Next** (bottom / right on the screen; reader closes, analysis program starts, it takes ca. 2-10 min dependent on the number of strips; reader takes images and automatically analyses the data). The progress of the reading is indicated by the following symbols:

photographed:  in analysis:  ready: 

- The reader indicates the end of the entire process with an acoustic signal (beep).
- Press the button **Next** (bottom / right on the screen; reader is opening).

- Remove the white frame with the ArrayStrip(s).
- Press the button **Next** (bottom / right on the screen; reader closes).

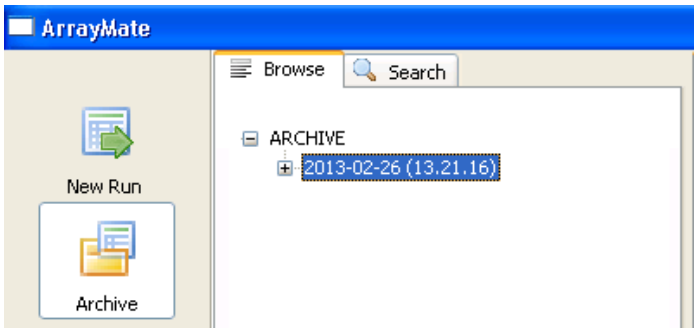
Results

On the lefthand side of the screen you will see a list showing all runs stored on the ArrayMate´s hard disk. A run contains the results from all arrays analysed together within one frame. If this list is not displayed:

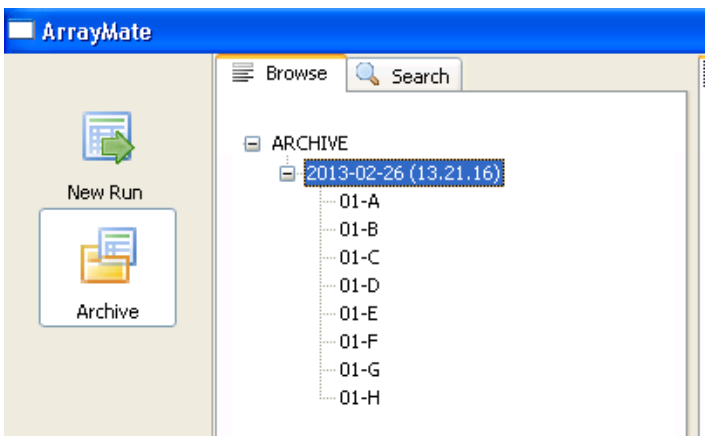
- Press the button **Archive** (lefthand) and activate the flag **Browse** (top left).

The runs are organised like folders in Windows Explorer, and **by default** named according to the date of acquisition.

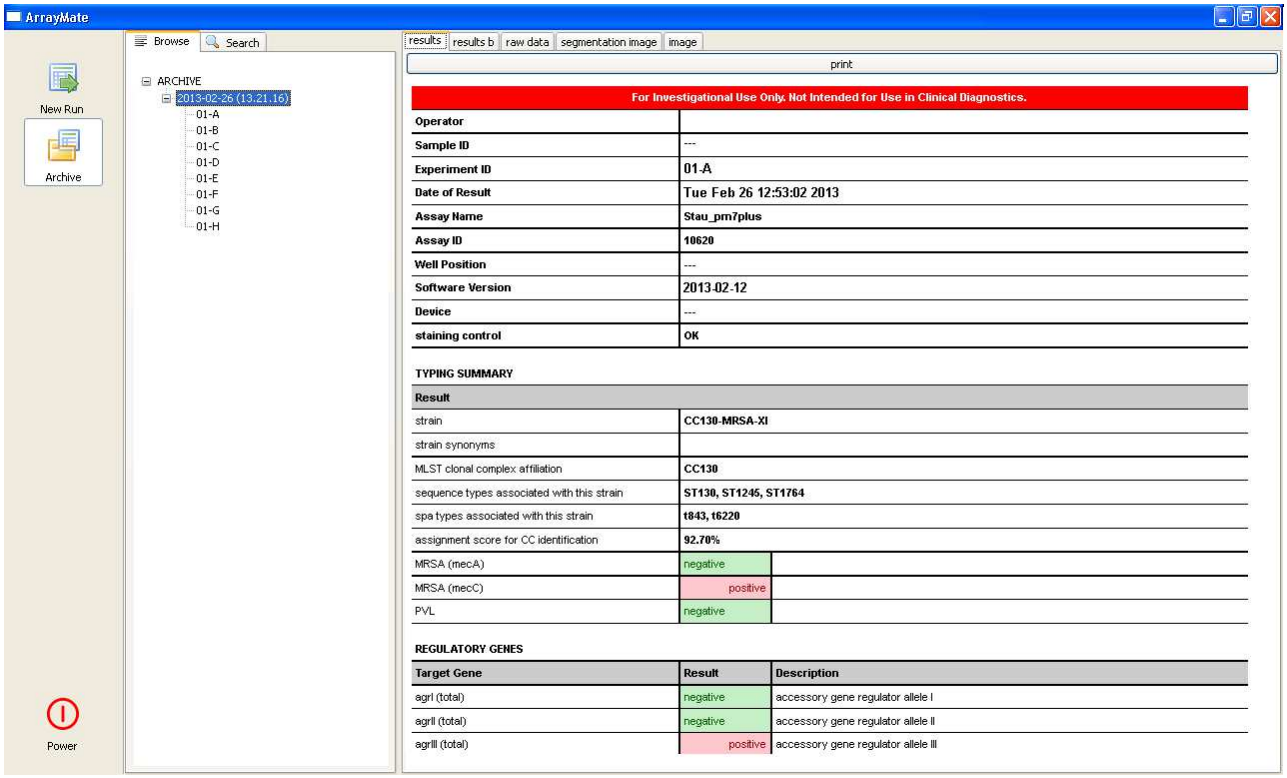
Example: There is one experiment run in this archive:



If you click on the plus symbol left on the run name, the folder opens and you will see a list of the individual arrays ordered by Sample ID.



Click on a Sample ID, and the *S. aureus* Genotyping Kit 2.0 test report for this array is shown in the window on the right:



Export of *S. aureus* Genotyping Kit 2.0 Test Reports

Two result files in htmL format will be generated. The shorter report will gives a summary on gene typing information.

This includes the clonal complex affiliation as derived from the overall hybridisation pattern and the strain affiliation as defined by clonal complex affiliation, presence / absence and type of SCC*mec* elements, and presence / absence of PVL or other relevant markers.

MLST sequence types and *spa* types known to be associated with this strain are also displayed. Note that this information is derived from a database search (see also Appendix 3), not from an actual experiment. Furthermore, results for virulence markers and genes associated to antibiotic resistance are listed.

A longer htmL result sheet (“result_B.res.htmL”) provides information on all probes.

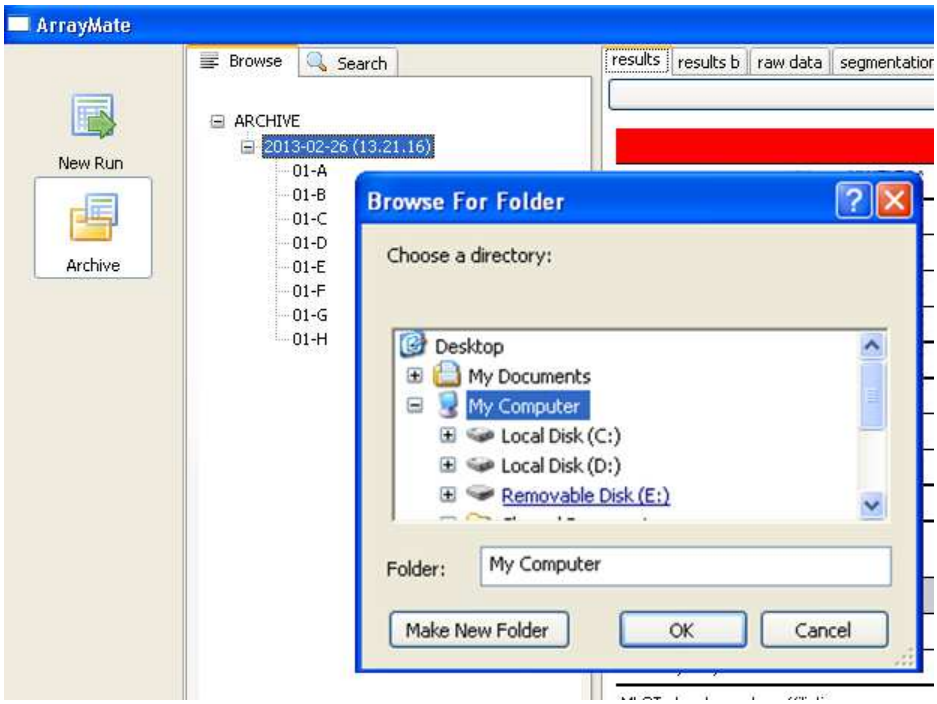
Possible error messages in these reports will be explained below (see Troubleshooting).

Other files that are generated and that can be exported include

- A *.txt file with the raw measurements,
- An image file (*.bmp) showing the actual photo of the array,
- A second image file (*.png) in which the coordinate grid is superimposed and the recognised spots are circled, and
- A *.xmL files providing contains the same information as the htmL result sheet for future export into databases etc.,
- An *.out file containing output log data which helps our service to trace image evaluation errors

Please Note: only complete runs can be exported. The export of individual S. aureus Genotyping 2.0 Test Reports is not possible

- Right-click on the selected run (a menu appears with the option **Export Run Reports**).
- Right-click on **Export Run Reports** (a file browser opens).



- Click on **My Computer**, subsequently on **Removable Disk,** and choose the folder where to save or click on the button **Make New Folder** (on the bottom; a new folder icon appears).

- Rename the new folder (e.g. with the experiment name or date).
- Click on the **OK** button (data are exported into the new folder on your memory stick).
- Do **NOT** remove the memory stick as long as the hourglass symbol is visible.
- Switch off the device by clicking on the **Power**-button (left / down on the screen): 

- Switch off the Screen. There is no need to physically switch off the ArrayMate Reader.

TROUBLESHOOTING

In case of trouble always make sure that reagents are within the recommended shelf-life and stored under appropriate conditions.

In case of trouble we are always happy to support. Please, contact cct.home@clondiag.com (or +49 (0) 3641 3111 0), and please include a description of the problem as well as the array images (*.bmp files) in question.

Please see Appendix 2 for sample images.

Staining Control

A staining control is included to check whether possible problems originate from the hybridisation or the staining procedure. If the staining control has “Failed” proceed as follows:

Horseradish peroxidase conjugate may have degraded during storage. Add 1 µL buffer C3/C4 to 9 µL D1 (substrate). If the solution turns green within 3-5 seconds, the horseradish peroxidase still has sufficient enzymatic activity.

Enzymatic reaction is inhibited by carryover of buffer C1. Ensure proper washing of the wells to remove all of Buffer C1 prior to adding horseradish peroxidase conjugate.

If the Staining Control has “Passed”, refer to the following hints.

Image Quality

In case of poor image quality we recommend to re-check DNA quantity and quality first by loading leftover DNA on an agarose gel.

In order to determine whether any problems originated from the DNA preparation, perform an experiment with the Control Material (CM). This is DNA from the reference strain N315 (GenBank accession number BA000018) and should be identified by the assay as “ST5-MRSA-II [tst1+], New York-Japan Clone”. If the control experiment yields a valid result and a correct identification, there was probably an issue with DNA preparation. If the control experiment also

fails as well, an error affecting later steps or a degradation of reagents applied in later steps is likely.

DNA Quality

The amount of DNA is crucial because of the linear kinetics of amplification (see Introduction). DNA should be free of RNA, as RNA reduces the efficiency of amplification and labelling by effectively removing primer from the reaction mix due to competitive hybridisation. A_{260} readings will cover RNA and other contaminants as well. Therefore pure DNA preparations without RNA contamination are prerequisite for proper DNA concentration measurement. RNAse treatment prior to A_{260} reading therefore is necessary (component A2 contains RNase).

DNA must be unfragmented, as fragmentation reduces the efficiency of amplification and labelling due to the distance between primer and probe binding sites. For this reason DNA should not be prepared by disrupting *S. aureus* cells using bead beaters, ultrasonication or aggressive chemicals such as in alkaline lysis protocols. We made good experiences with the manual QIAGEN DNeasy Kit and the automated device EZ1.

DNA must be free of any trace of ethanol, as ethanol strongly influences the amplification. It is possible to heat the sample prior to adding it to the labelling mix (5-10 min at 70 °C). Some problems with samples from the Qiagen EZ1 device for example were resolved after heating the samples (see above).

Physical Damage to the Array

Scratching of the array surface with a pipette tip can lead to damage of array spots which in turn prohibits the acquisition of a valid signal. In this case the respective marker is not assigned as “negative”, but instead the message “none” appears next to the marker name.

Ambiguous Results

Apart from a “positive” or “negative” result for the individual markers on the *S. aureus* Genotyping Test Report, the result can also be “ambiguous”.

In cases affecting resistance genes or virulence factors, no definitive answer with regard to this specific marker can be given. This can be caused by poor sample quality, poor signal quality and, especially in some resistance-associated genes such *aacA-aphD*, by the presence of plasmids in low copy numbers.

Please note, that for some markers, for which allelic variants were to be discriminated (*bbp, clfA, clfB* and *fnbB* as well as some *set/ssl* genes*, isaB, mprF* and *isdA*), a different approach for analysis was used than for resistance genes or virulence factors. In these genes, alleles that differ only in single nucleotides are recognised. For the sake of creation of identifiable clonal complex-specific patterns, only the probe with the strongest signal value is regarded as positive, provided that it exceeded a defined breakpoint. All other allele-specific probes are then regarded as ambiguous or, if below the breakpoint, as negative. Therefore it is perfectly normal, if a number of allele-specific probes for these genes yield "ambiguous" signals. The presence or absence of these genes is indicated by fields such as, e.g. "*clfA* (total)" which are summaries for all probes related to the respective gene.

Report Unavailable

If the ArrayMate indicates that no report is available for an array (or multiple arrays on one strip), please check that the strip was positioned properly into the frame. Scratches or drops of condensed water might render the Data Matrix code identifier unreadable, please wipe it carefully or try to manually identify the test.

If no obvious reason for the fault can be discovered, please contact the technical service.

Error Messages in Result Sheets

Please compare Appendix 2 for images. If strains cannot be identified, error messages are displayed instead of the short htmL result sheet. In order to facilitate searching for the cause of the error, the long ("result_B.res.htmL") htmL result sheet will be generated although it might be faulty. However, it might give a hint what the cause of the problem was.

One possible error message is: "*Identification is not possible. This could be due to technical issues such as poor signal quality, overstaining or to contamination. Please re-clone the culture, confirm its identity as Staphylococcus aureus and its purity, and repeat the experiment. Identification is also not possible for strains that are not covered by the database. If this is likely (i.e., if your isolate is repeatedly un-identifiable or if you have additional typing data suggesting an unknown strain), please submit the array image and/or the isolate in question to Alere Technologies*." This will appear for instance when the pattern is entirely irregular or if mutually exclusive alleles are detected simultaneously. The long ("result_B.res.htmL") htmL result sheets might show in the latter case that several *agr* types or capsule types 5 and 8 were detected in one sample. This can be caused by massive unspecific staining or by contamination / mixed culture. Re-clone and repeat. If this message was prompted by a technically faultless experiment, and if contamination can be ruled out by repeated cloning, please submit the picture and/or the strain for further analysis. It might be an unknown strain that cannot be identified because it was not included into the database. If this is the case we will use multilocus sequence typing (MLST) for further characterisation and might include this strain into future database updates.

This error message in the short result sheet accompanied by positive signals *only* for resistance and SCC*mec* associated genes indicates the presence of different staphylococcal species (*Staph. epidermidis, Staph. haemolyticus etc.*). The long ("result_B.res.htmL") htmL result sheet should provide this information, occasionally a faulty identification as "*Staph. argenteus*" lineage (CC75), albeit at a low score, might occur.

Another error message "*An assignment to a strain is not possible, although the clonal complex was recognised. This might be caused by technical issues such as poor signal quality, overstaining or contamination. The isolate could also represent a new strain within a known clonal complex, i.e., a strain carrying an unusual SCCmec element or an unusual set of virulence genes. If this appears to be the case, please submit the array image and/or the isolate in question to Alere Technologies*" might appear instead of the typing information in an otherwise normal result file. This could indicate an unusual SCC*mec* element or an unusual presence of virulence genes, such as of PVL in a lineage where it has not been observed before. A contamination, e.g. by SCC-bearing coagulase-negatives, needs to be ruled out. Re-clone and

repeat. If this message was prompted by a technically faultless experiment, and if contamination can be ruled out by repeated cloning, please submit the picture and/or the strain for further analysis.

ADDITIONAL INFORMATION

Warranty

Alere Technologies GmbH guarantees the performance as described in this manual. Usage of the Kit was successfully tested at ambient temperatures up to 37 °C, a guarantee is limited to ambient temperatures in the laboratory between 18-28 °C. Kit components comprise the arrays and their caps, the Lysis Enhancer, the reagents for DNA labelling and for detection of labelled DNA products on the array, the ArrayMate Reader and its software. In case one of these components fails within the expiry date due to other reason than misuse, contact Alere Technologies GmbH for replacement or refund. Terms and conditions apply.

If you have any problem or question, please contact the technical service.

Disclaimer

This system is for research use only.

We do not accept any liability for damages caused by misuse. Misuse comprises, especially but not exclusively, of a use of the system for the detection of resistance genes in order to predict phenotypic antibiotic resistances or susceptibilities for the guidance of an antibiotic chemotherapy.

Since resistances might be caused by genes or mutations not covered by this array or by hitherto unknown genes or mutations, any antibiotic chemotherapy MUST be guided by phenotypic susceptibility tests.

Furthermore, we do not accept any liability for damages caused by inappropriate use of the device as a personal computer, for instance related to the use of additional software, to network connections, or to a breach of privacy related to the storage of confidential

information (such as names of patients from whom *S. aureus* was isolated) on its hard disk and/or to the use of external storage devices that might be contaminated with spyware.

Quality Control

Each batch is stringently tested with the use of standard *S. aureus* DNA preparations for good performance and correctness of results.

List of Components for Separate Order

If required, these reagents for the *S. aureus* Genotyping Kit 2.0 may be ordered separately:

Component	Name	Amount	Cat#	Storage
A1	Lysis Buffer	30 mL	245101000	18-28 °C
A2	Lysis Enhancer	96 units	245102000	18-28 °C
$B1^{ST2}$	Labelling Buffer / Master Mix	700 µL	245203000	2-8 °C
B2	Labelling Enzyme	20 µL	245104000	2-8 °C
C1	Hybridisation Buffer	30 mL	245105000	18-28 °C
C2	Washing Buffer 1	120 mL	245106000	18-28 °C
C3	HRP Conjugate 100x	200 µL	245107000	2-8 °C
C4	Conjugate Buffer	30 mL	245108000	18-28 °C
C5	Washing Buffer 2	120 mL	245109000	18-28 °C
D1	HRP Substrate	15 mL	245110000	2-8 °C
ArrayStrips	ArrayStrip *S. aureus* 2.0	12 St	240009601	15-28 °C
StripCaps	Covers for unused arrays	24 St	245112000	18-28 °C
CM	Control Material (N315 DNA)	30 µL	245111000	2-8 °C
-	CC130-MRSA-XI DNA, (*mecC* positive)	30 µL	Can be provided upon request	2-8 °C

For pricing please contact your local representative or our customer service, respectively.

Legal Manufacturer

Alere Technologies GmbH

Loebstedter Str. 103-105

07749 Jena, Germany

www.clondiag.com
www.alere-technologies.com

Contact

If you require any further information on this product please contact cct.home@clondiag.com

LITERATURE

Literature quoted in this manual:

[1] Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, Chow H, Ip M, Jatzwauk L, Jonas D, Kadlec K, Kearns A, Laurent F, O'Brien FG, Pearson J, Ruppelt A, Schwarz S, Scicluna E, Slickers P, Tan H-L, Weber S, Ehricht R (2011) A Field Guide to Pandemic, Epidemic and Sporadic Clones of Methicillin-Resistant *Staphylococcus aureus.* PLoS One 6 (4):e17936

[2] Monecke S, Slickers P, Ehricht R (2008) Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. FEMS Immunol Med Microbiol 53:237–251

[3] Garcia-Alvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA (2011) Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 11 (8):595-603

[4] Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, Ehricht R, Coleman DC (2011) Detection of staphylococcal cassette chromosome *mec* type XI carrying highly divergent *mecA, mecI, mecR1, blaZ,* and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus.* Antimicrob Agents Chemother 55 (8):3765-3773

For further literature please refer to:

http://alere-technologies.com/en/science-technologies/publications/saureus.htmL

UPDATES AND SOFTWARE

Notifications on database/software updates and freeware tools can be found at:

http://alere-technologies.com/en/science-technologies/publications/downloads.htmL.

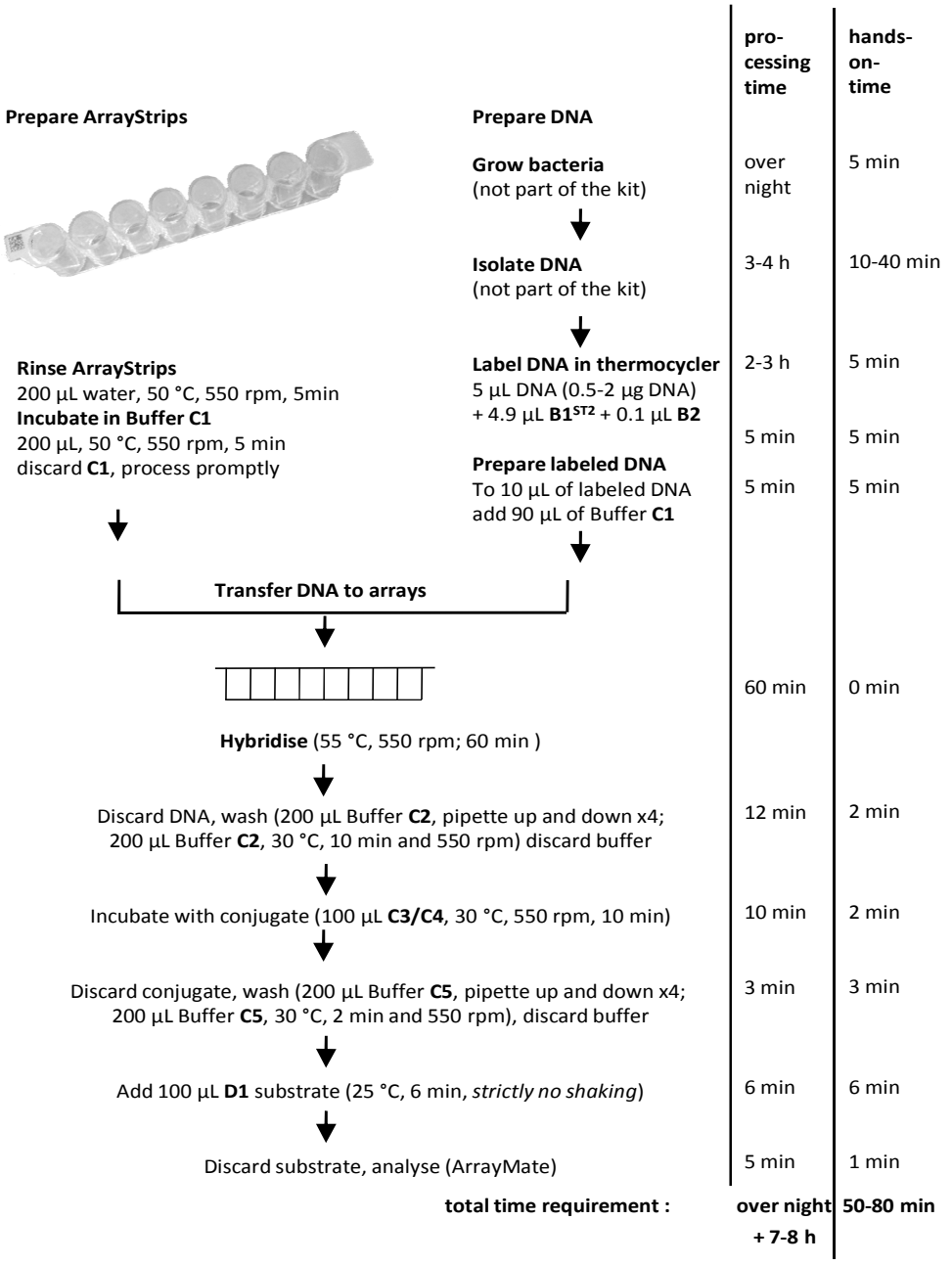
and/or http://alere-technologies.com/en/news.htmL.

Currently available freeware programs are:

- “spa type mapper” for the analysis of spa sequences
- “Alere S aureus Results Collector” for the conversion of multiple *result.xmL files from the ArrayMate into spreadsheet tables. This should make it easier to compare isolates or to determine relative abundances of genes or strains etc.

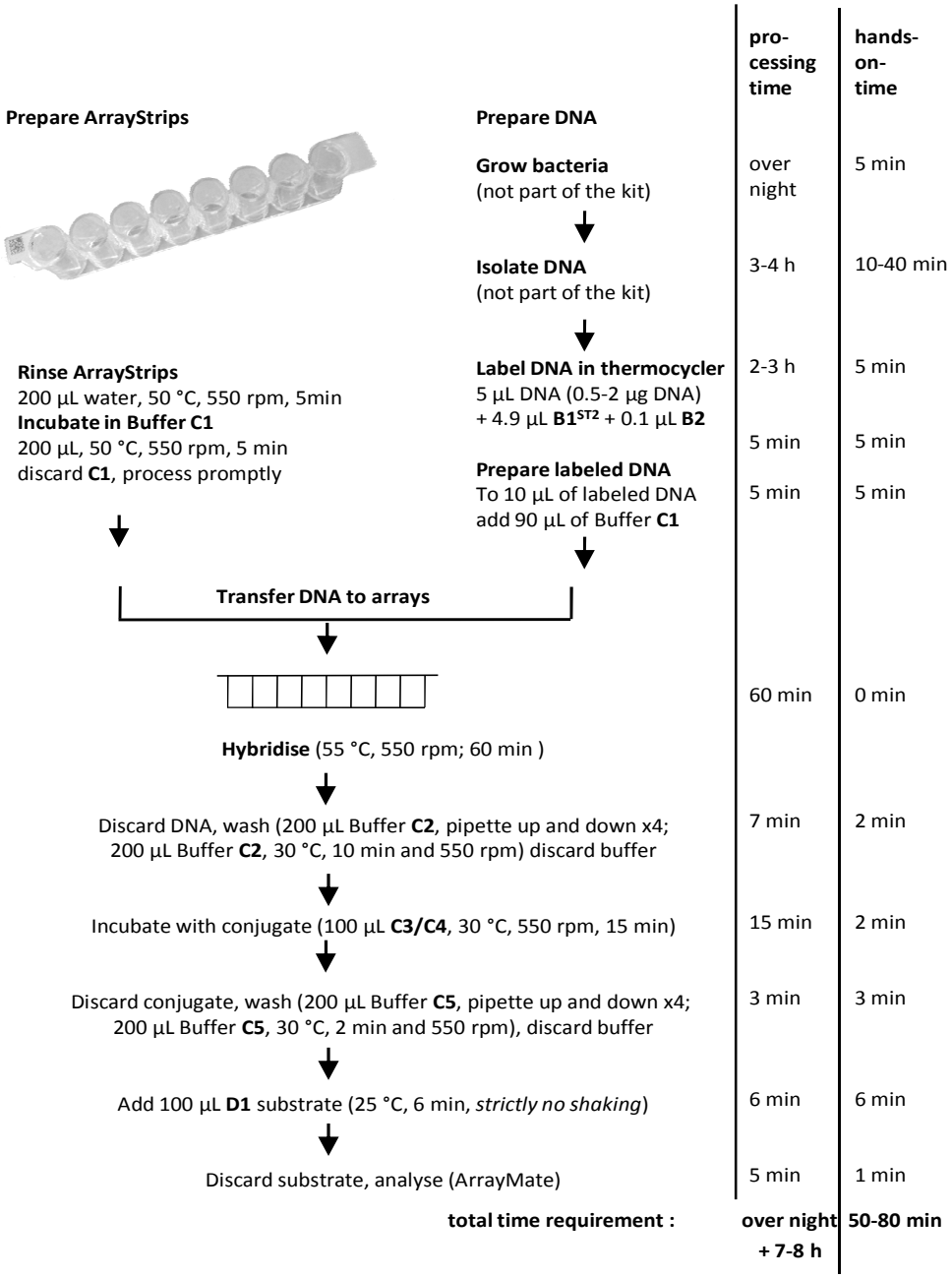
APPENDIX 1 - FLOW CHART

Quantifoil protocol. The figure on this page summarises the test procedure for the thermoshaker BioShake iQ by Quantifoil. Please always refer to the text section of this manual for further important details.

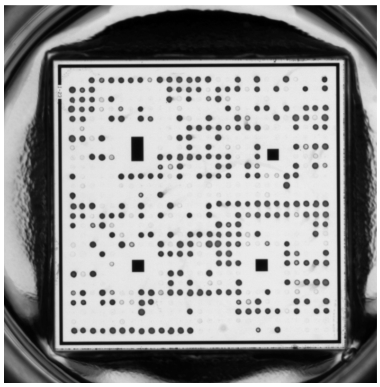
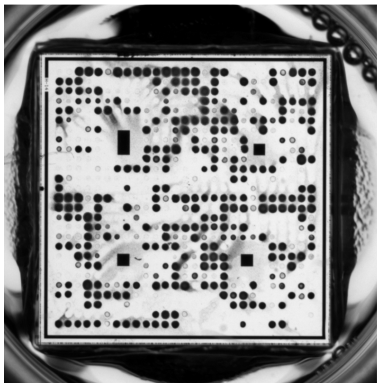
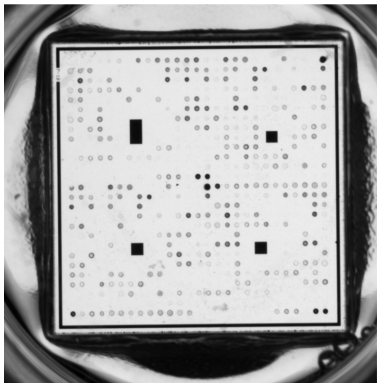
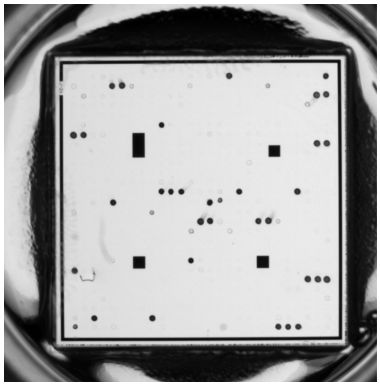


APPENDIX 1 - FLOW CHART

Eppendorf protocol. The figure on this page summarises an adapted test procedure for the thermoshaker Thermomixer Comfort by Eppendorf. Please always refer to the text section of this manual for further important details.



APPENDIX 2 – IMAGES FOR TROUBLESHOOTING

Image	Comment	Result sheets:
	A technically faultless, valid experiment.	Valid results, no error messages.
	This image is overstained. The experiment should be repeated.	There might be no error messages although individual probes might yield false-positives. The error message "*An assignment to a strain is not possible, although the clonal complex was recognised. This might be caused by technical issues such as poor signal quality, overstaining or contamination.* …" might appear, if false-positives hinder strain identification.
	This image is poor. This could be due to low DNA concentration, fragmented DNA, ethanol trace contaminations in DNA sample or expired reagents. The experiment should be repeated with a new DNA preparation. If this also fails, try an experiment with N315 control DNA (CM).	The error message "*An assignment to a strain is not possible, although the clonal complex was recognised. This might be caused by technical issues such as poor signal quality, overstaining or contamination. The isolate could also represent a new strain within a known clonal complex* …" might appear in the short file. The long file will yield an approximate identification, but individual probes might yield false-negative results.
	Species other than *S. aureus* tested. Check identification by other means.	Error message in the short htmL file. The long file yields "*Coagulase-negative Staphylococci, other bacteria, or very poor signal quality. Check identification by biochemical means or MALDI-TOF or repeat experiment*" and shows positive results only for resistance genes and/or genes associated with SCC*mec*.

APPENDIX 3 – TARGET GENES

SPECIES MARKER	domain 1 of 23S-rRNA	*rrnD1 (S. aureus)*
	glyceraldehyde 3-phosphate dehydrogenase, locus 1	*gapA*
	katalase A	*katA*
	coagulase	*coA*
	thermostable extracellular nuclease	*nuc1*
	staphylococcal protein A	*spa*
	IgG-binding protein	*sbi*
REGULATORY GENES	staphylococcal accessory regulator A	*sarA*
	histidine protein kinase, sae locus	*saeS*
	sensor protein	*vraS*
	accessory gene regulator allele I	*agrI (total)*
		agrB-I
		agrC-I
		agrD-I
	accessory gene regulator allele II	*agrII (total)*
		agrB-II
		agrC-II
		agrD-II
	accessory gene regulator allele III	*agrIII (total)*
		agrB-III
		agrC-III
		agrD-III
	accessory gene regulator allele IV	*agrIV (total)*
		agrB-IV
		agrC-IV
	haemolysin delta	*hld*
METHICILLIN RESISTANCE AND SCCmec TYPING	alternate penicillin binding protein 2, defining MRSA	*mecA*
	novel mecA homologue, also associated with beta lactam resistance	***mecC***
	truncated signal transducer protein MecR1	*delta_mecR*
	glycerophosphoryl diester phosphodiesterase, associated with mecA	*ugpQ*
	cassette chromosome recombinase genes A-1	*ccrA-1*
	cassette chromosome recombinase genes B-1	*ccrB-1*
	plasmin-sensitive surface protein	*plsSCC (COL)*
	hypothetical protein from SCCmec elements	*Q9XB68-dcs*
	cassette chromosome recombinase gene A-2	*ccrA-2*
	cassette chromosome recombinase gene B-2	*ccrB-2*
	potassium-translocating ATPase A, chain 2	*kdpA-SCC*
	potassium-transporting ATPase B, chain 1	*kdpB-SCC*
	potassium-translocating ATPase C, chain 2	*kdpC-SCC*
	sensor kinase protein	*kdpD-SCC*
	KDP operon transcriptional regulatory protein	*kdpE-SCC*
	methicillin-resistance gene regulatory protein	*mecI*
	signal transducer protein MecR1	*mecR*
	homolog of xylose repressor, associated with SCCmec-	*xylR*

	elements	
	cassette chromosome recombinase gene A-3	*ccrA-3*
	cassette chromosome recombinase gene B-3	*ccrB-3*
	mercury resistance gene operon, Hg(II) reductase	*merA*
	mercury resistance gene operon, alkylmercury lyase	*merB*
	Putative protein, homologue to cassette chromosome recombinase A genes	*ccrAA (MRSAZH47)_probe 1*
		ccrAA (MRSAZH47)_probe 2
	cassette chromosome recombinase gene C	*ccrC (85-2082)*
	cassette chromosome recombinase gene A-4	*ccrA-4*
	cassette chromosome recombinase gene B-4	*ccrB-4*
RESISTANCE : PENICILLINASE	beta-lactamase gene	*blaZ*
	beta-lactamase gene associated with SCCmec XI elements	***blaZ-SCCmec XI***
	beta lactamase repressor (inhibitor)	*blaI*
	beta-lactamase regulatory protein	*blaR*
RESISTANCE : MLS-ANTIBIOTICS	rRNA methyltransferase associated with macrolide/lincosamide resistance	*erm(A)*
	rRNA methyltransferase associated with macrolide/lincosamide resistance	*erm(B)*
	rRNA methyltransferase associated with macrolide/lincosamide resistance	*erm(C)*
	lincosaminide nucleotidyltransferase (=linA)	*lnu(A)*
	macrolide efflux pump	*msr(A)*
	macrolide efflux protein A	*mef(A)*
	macrolide phosphotransferase II (=mpbBM)	*mph(C)*
	virginiamycin A acetyltransferase	*vat(A)*
	acetyltransferase inactivating streptogramin A	*vat(B)*
	ABC transporter conferring resistance to streptogramin A and related compounds	*vga(A)*
	vga(A) allele from strain BM 3327	*vga(A) (BM 3327)*
	virginiamycin B hydrolase (=vgb)	*vgB(A)*
RESISTANCE : AMINOGLYSOSIDES	aminoglycoside adenyl-/phosphotransferase (gentamicin, tobramycin)	*aacA-aphD*
	aminoglycoside adenyltransferase (neo-/ kanamycin, tobramycin)	*aadD*
	aminoglycoside phosphotransferase (neo-/ kanamycin)	*aphA3*
RESISTANCE : MISCELLANEOUS GENES	streptothricin acetyltransferase	*sat*
	dihydrofolate reductase mediating trimethoprim resistance (=dfrA)	*dfrS1*
	fusidic acid resistance gene (= far1)	*fusB*
	fusidic acid resistance gene (= Q6GD50)	*fusC*
	isoleucyl-tRNA synthetase associated with mupirocin resistance (=mupR)	*mupA*
	tetracycline efflux protein	*tet(K)*
	ribosomal protection protein associated with tetracycline resistance	*tet(M)*
	chloramphenicol acetyltransferase	*cat (total)*
		cat (pC221)
		cat (pc223)
		cat (pMC524)

		cat (pSBK203R)
	23S rRNA methyltransferase (phenicols, lincosamides, oxazolidinones, pleuromutilins, streptogramin A)	*cfr*
	chloramphenicol/florfenicol exporter	*fexA*
	metallothiol transferase	*fosB*
		fosB (plasmid)
RESISTANCE : EFFLUX SYSTEMS	quaternary ammonium compound / multidrug efflux protein C	*qacA*
	quaternary ammonium compound / multidrug efflux protein A	*qacC (total)*
		qacC (consensus)
		qacC (equine)
		qacC (SA5)
		qacC (Ssap)
		qacC (ST94)
	putative transport protein (=tetEfflux)	*sdrM*
RESISTANCE : GLYCOPEPTIDES	vancomycin resistance gene	*vanA*
	vancomycin resistance gene from enterococci and Clostridium	*vanB*
	teicoplanin resistance gene from enterococci	*vanZ*
VIRULENCE : TOXIC SCHOCK TOXIN	toxic shock syndrome toxin 1	*tst1 (consensus)*
		tst1 ("human" allele)
		tst1 ("bovine" allele, from RF122)
VIRULENCE : ENTEROTOXINS	enterotoxin A (=entA)	*sea*
	enterotoxin A, allele from strain 320E	*sea (320E)*
	enterotoxin A, allele from strain N315 = enterotoxin P	*sea (N315)*
	enterotoxin B (=entB)	*seb*
	enterotoxin C (=entC)	*sec*
	enterotoxin D (=entD)	*sed*
	enterotoxin E (=entE)	*see*
	enterotoxin G (=entG)	*seg*
	enterotoxin H (=entH)	*seh*
	enterotoxin I (=entI)	*sei*
	enterotoxin J (=entJ)	*sej*
	enterotoxin K (=entK)	*sek*
	enterotoxin L (=entL)	*sel*
	enterotoxin-like gene/protein M (=sem, entM)	*selm*
	enterotoxin-like gene/protein N (=sen, entN)	*seln (consensus)*
		seln (other than RF122)
	enterotoxin-like gene/protein O (=seo, entO)	*selo*
	enterotoxin gene cluster (seg/i/selm/n/o/u)	*egc*
	enterotoxin Q (=entQ)	*seq*
	enterotoxin R (=entR)	*ser*
	enterotoxin-like gene/protein U (=seu, entU)	*selu*
	enterotoxin-like protein ORF CM14	*ORF CM14_ probe1*
	enterotoxin-like protein ORF CM14	*ORF CM14_ probe2*
VIRULENCE : HLG AND LEUKOCIDINS	haemolysin gamma / leukocidin, component B (F)	*lukF*
	haemolysin gamma / leukocidin, component C (S)	*lukS*

	haemolysin gamma / leukocidin, component C (S), allele from ST22 and ST45	*lukS (ST22+ST45)*
	haemolysin gamma, component A	*hlgA*
	Panton Valentine leukocidin F component	*lukF-PV*
	Panton Valentine leukocidin S component	*lukS-PV*
	F component of leukocidin from ruminants	*lukF-PV (P83)*
	S component of leukocidin from ruminants	*lukM*
	leukocidin D component	*lukD*
	leukocidin E component	*lukE*
	leukocidin/ haemolysin toxin family protein	*lukX*
	leukocidin/haemolysin toxin family protein, allele from ST30 and ST45	*lukY*
	leukocidin/haemolysin toxin family protein	*lukY (ST30+ST45)*
VIRULENCE : HAEMOLYSINS	putative membrane protein	*hl*
	haemolysin alpha	*hla*
	putative membrane protein	*hlIII (consensus)*
		hlIII (other than RF122)
	haemolysin beta	*hlb_probe 1*
	haemolysin beta	*hlb_probe 2*
	haemolysin beta	*hlb_probe 3*
	haemolysin beta without phage insertion	*un-disrupted hlb*
VIRULENCE : HLB-CONV PHAGES	staphylokinase	*sak*
	chemotaxis-inhibiting protein (CHIPS)	*chp*
	staphylococcal complement inhibitor	*scn*
VIRULENCE : EXFOLIATIVE TOXINS	exfoliative toxin serotype A	*etA*
	exfoliative toxin serotype B	*etB*
	exfoliative toxin D	*etD*
VIRULENCE : EPITHEL. DIFF. INHIB	epidermal cell differentiation inhibitor	*edinA*
	epidermal cell differentiation inhibitor B	*edinB*
	epidermal cell differentiation inhibitor C	*edinC*
VIRULENCE : ACME LOCUS	Arginine Catabolic Mobile Element	*ACME cluster*
	ACME-locus: arginine deiminase	*arcA-SCC*
	ACME-locus: ornithincarbamoyltransferase	*arcB-SCC*
	ACME-locus: carbamatkinase	*arcC-SCC*
	ACME-locus: arginine/ornithine-antiporter	*arcD-SCC*
VIRULENCE : PROTEASES	aureolysin	*aur (consensus)*
		aur (other than MRSA252)
		aur (MRSA252)
	serinprotease A	*splA*
	serinprotease B	*splB*
	serinprotease E	*splE*
	glutamylendopeptidase	*sspA*
	staphopain B, protease	*sspB*
	staphopain A (staphylopain A), protease	*sspP (consensus)*
		sspP (other than ST93)
VIRULENCE : STAPHYLOCOCCAL SUPERANTIGEN/ ENTEROTOXIN-LIKE	staphylococcal exotoxin-like protein/SAg gene homolog, SAUSA300_0370	*setC/selx*
	staphylococcal superantigen-like protein 1 (probes)	*ssl01/set6_probe1_11*
		ssl01/set6_probe2_11

GENES (SET/SSL)		*ssl01/set6_probe1_12*
		ssl01/set6_probe2_12
		ssl01/set6_probe4_11
		ssl01/set6_probeRF122
	staphylococcal superantigen-like protein 1 (interpretation/alleles)	*ssl01/set6 (COL)*
		ssl01/set6 (Mu50+N315)
		ssl01/set6 (MW2+MSSA476)
		ssl01/set6 (MRSA252)
		ssl01/set6 (RF122)
		ssl01/set6 (other alleles)
	staphylococcal superantigen-like protein 2	*ssl02/set7*
		ssl02/set7 (MRSA252)
	staphylococcal superantigen-like protein 3	*ssl03/set8_probe 1*
		ssl03/set8_probe 2
		ssl03/set8 (MRSA252, SAR0424)
	staphylococcal superantigen-like protein 4	*ssl04/set9*
		ssl04/set9 (MRSA252, SAR0425)
	staphylococcal superantigen-like protein 5	*ssl05/set3_probe 1*
		ssl05/set3 (RF122, probe-611)
		ssl05/set3_probe 2 (612)
		ssl05/set3 (MRSA252)
	staphylococcal superantigen-like protein 6	*ssl06/set21*
		ssl06 (NCTC8325+MW2)
	staphylococcal superantigen-like protein 7	*ssl07/set1*
		ssl07/set1 (MRSA252)
		ssl07/set1 (AF188836)
	staphylococcal superantigen-like protein 8	*ssl08/set12_probe 1*
		ssl08/set12_probe 2
	staphylococcal superantigen-like protein 9	*ssl09/set5_probe 1*
		ssl09/set5_probe 2
		ssl09/set5 (MRSA252)
	staphylococcal superantigen-like protein 10	*ssl10/set4*
		ssl10 (RF122)
		ssl10/set4 (MRSA252)
	staphylococcal superantigene-like protein 11	*ssl11/set2 (COL)*
		ssl11+set2(Mu50+N315)
		ssl11+set2(MW2+MSSA476)
		ssl11/set2 (MRSA252)
	staphylococcal exotoxin-like protein, second locus	*setB3*
		setB3 (MRSA252)
		setB2
		setB2 (MRSA252)
		setB1
CAPSULE- AND BIOFILM-ASSOCIATED GENES	capsule type 1	*cap 1 (total)*
	capsular polysaccharide synthesis enzyme	*capH1*

	O-antigen polymerase	*capJ1*
	capsular polysaccharide biosynthesis protein	*capK1*
	capsule type 5	*cap 5 (total)*
	capsular polysaccharide synthesis enzyme	*capH5*
	O-antigen polymerase	*capJ5*
	capsular polysaccharide biosynthesis protein	*capK5*
	capsule type 8	*cap 8 (total)*
	capsular polysaccharide synthesis enzyme	*capH8*
	capsular polysaccharide biosynthesis protein	*capI8*
	O-antigen polymerase	*capJ8*
	capsular polysaccharide biosynthesis protein	*capK8*
	intercellular adhesion protein A	*icaA*
	intercellular adhesion protein C	*icaC*
	biofilm PIA synthesis protein D	*icaD*
	surface protein involved in biofilm formation	*bap*
ADHAESION FACTORS / GENES ENCODING MICROBIAL SURFACE COMPONENTS RECOGNIZING ADHESIVE MATRIX MOLECULES (MSCRAMM GENES)	bone sialoprotein-binding protein	*bbp (total)*
		bbp (consensus)
		bbp (COL+MW2)
		bbp (MRSA252)
		bbp (Mu50)
		bbp (RF122)
		bbp (ST45)
	clumping factor A	*clfA (total)*
		clfA (consensus)
		clfA (COL+RF122)
		clfA (MRSA252)
		clfA (Mu50+MW2)
	clumping factor B	*clfB (total)*
		clfB (consensus)
		clfB (COL+Mu50)
		clfB (MW2)
		clfB (RF122)
	collagen-binding adhesin	*cna*
	cell wall associated fibronectin-binding protein	*ebh (consensus)*
	cell surface elastin binding protein	*ebpS (total)*
		ebpS_probe 612
		ebpS_probe 614
		ebpS (01-1111)
		ebpS (COL)
	enolase	*eno*
	fibrinogen binding protein (19 kDa)	*fib*
		fib (MRSA252)
	fibronectin-binding protein A	*fnbA (total)*
		fnbA (consensus)
		fnbA (COL)
		fnbA (MRSA252)
		fnbA (Mu50+MW2)
		fnbA (RF122)

	fibronectin-binding protein B	*fnbB (total)*
		fnbB (COL)
		fnbB (COL+Mu50+MW2)
		fnbB (Mu50)
		fnbB (MW2)
		fnbB (ST15)
		fnbB (ST45-2)
	major histocompatibility complex class II analog protein (=Extracellular adherence protein, eap)	*map (total)*
		map (COL)
		map (MRSA252)
		map (Mu50+MW2)
	Staphylococcus aureus surface protein G	*sasG (total)*
		sasG (COL+Mu50)
		sasG (MW2)
		sasG (other than MRSA252+RF122)
	Ser-Asp rich fibrinogen-/ bone sialoprotein-binding protein C	*sdrC (total)*
		sdrC (consensus)
		sdrC (B1)
		sdrC (COL)
		sdrC (Mu50)
		sdrC (MW2+MRSA252+RF122)
		sdrC (other than MRSA252+RF122)
	Ser-Asp rich fibrinogen-/ bone sialoprotein-binding protein D	*sdrD (total)*
		sdrD (consensus)
		sdrD (COL+MW2)
		sdrD (Mu50)
		sdrD (other)
	van Willebrand factor binding protein	*vwb (total)*
		vwb (consensus)
		vwb (COL+MW2)
		vwb (MRSA252)
		vwb (Mu50)
		vwb (RF122)
IMMUNODOMINANT ANTIGEN B	immunodominant antigen B	*isaB*
		isaB (MRSA252)
DEFENSIN RESISTANCE	defensin resistance gene protein	*mprF (COL+MW2)*
		mprF (Mu50+MRSA252)
TRANSFERRIN BINDING PROT	transferrin-binding protein	*isdA (consensus)*
		isdA (MRSA252)
		isdA (other than MRSA252)
PUTATIVE TRANSPORTER	hypothetical protein, similar to integral membrane protein LmrP	*lmrP (other than RF122)_probe1*
		lmrP (other than RF122)_probe2
		lmrP (RF122)_probe1
		lmrP (RF122)_probe2

TYPE I RESTRICTION-MODIFICATION SYSTEM, SINGLE SEQUENCE SPECIFICITY PROTEIN	type I site-specific deoxyribonuclease subunit, 1st locus	*hsdS1 (RF122)*
	type I site-specific deoxyribonuclease subunit, 2nd locus	*hsdS2 (Mu50+N315+COL+USA300+ NCTC8325)*
		hsdS2 (MW2+MSSA476)
		hsdS2 (RF122)
		hsdS2 (MRSA252)
	type I site-specific deoxyribonuclease subunit, 3rd locus	*hsdS3 (all other than RF122+ MRSA252)*
		hsdS3 (COL+USA300+NCTC8325+MW2+MSSA476+RF122)
		hsdS3 (Mu50+N315)
		hsdS3 (CC51+ MRSA252)
		hsdS3 (MRSA252)
	type I site-specific deoxyribonuclease subunit, unknown locus	*hsdSx (CC25)*
		hsdSx (CC15)
		hsdSx (etd)
MISCELLANEOUS GENES	hypothetical protein, located next to serine protease operon	*Q2FXC0*
	unspecific efflux/transporter	*Q2YUB3*
	hypothetical protein	*Q7A4X2*
HYALURONATE LYASE	hyaluronate lyase, first / second locus	*hysA1 (MRSA252)*
		hysA1 (MRSA252+RF122) and/or hysA2 (consensus)
		hysA1 (MRSA252+RF122) and/or hysA2 (COL+USA300)
	hyaluronate lyase, second locus	*hysA2 (all other than MRSA252)*
		hysA2 (COL+USA300+NCTC8325)
		hysA2 (all other than COL+USA300+NCTC8325)_ probe1
		hysA2 (all other than COL+USA300+NCTC8325)_ probe1
		hysA2 (MRSA252)

NOTE: “(consensus)” indicates true consensus probes while “(total)” indicates a summary for all probes for a given gene to show on one glance whether this gene is present, in any known allele, or not.

APPENDIX 4 – TYPING INFORMATION

Definitions and Explanations

The displayed result will yield following typing information:

- Strain assignment, as determined by overall profile and by preset definitions for strains. Strains are always defined by clonal complex affiliation (see below), absence or presence of *mecA* and by SCC*mec* type as well as absence or presence of PVL. Widely or historically recognised strains might also be defined based on the absence or presence of additional characteristic genes.
- Strain synonyms. These are listed if they can be unambiguously attached to strains as defined above. If you use local designations for strains that you want to be included, please contact stefan.monecke@clondiag.com
- MLST clonal complex affiliation, as determined by overall profile and by preset definitions including capsule types and *agr* groups. Isolates can be assigned to clonal complexes as defined by multilocus sequence typing. Analysis of hybridisation patterns cannot discriminate sequence types which differ only in single point mutations affecting MLST genes (e.g., ST5 and ST225, or ST59 and ST952). However, there are also sequence types which originate from chromosomal replacements. Examples are CC8/ST239 or CC30/ST34. As these events result in different hybridisation patterns, such STs can be easily identified. Some other STs are also clearly different from parental CCs although recombination is not (yet?) proven. In such cases, ST affiliation might also be displayed (for instance for CC9/ST834)
- Sequence types associated with this strain. This information is provided based on a database of isolates that have been typed in parallel by array and MLST. It is not directly derived from the actual experiment. If you have MLST results you want to be included, please contact stefan.monecke@clondiag.com.
- *spa* types associated with this strain. This information is provided based on a database of isolates that have been typed in parallel by array and *spa* sequencing. It is not directly

derived from the actual experiment. If you have *spa* results you want to be included, please contact stefan.monecke@clondiag.com.

- Assignment score. This is a score for the similarity to the average hybridisation result for a given strain/CC. Scores below 88% exclude reliable strain identification, and could be attributed either to technical reasons or to the presence of a yet unknown strain. The "short" htmL file will display an error message; the "long" htmL file will display the most similar strain although that identification might be faulty. A value of 100% is unlikely because of the mobility of many genes in *S. aureus.*

List of Currently Recognised Strains

If you have array images of a strain not yet covered or if you have additional information on strain you wish to be included, such as local synonyms, spa types or MLST types, please contact stefan.monecke@clondiag.com.

MLST CC	Strain	Synonymes	MLST types associated with this strain	spa types associated with
CC1	**CC1-MSSA**		ST1, ST761, ST762	t127, t174, t398, t559, t1506, t6980
	CC1-MSSA [lukF-P83/lukM+]			
	CC1-MSSA [PVL+]		ST1	t174
	CC1-MSSA-SCCfus	Sanger MSSA476	ST1	t607, t559, t2246, t8989
	CC1-MSSA-SCCfus [PVL+]		ST1	t127, t177, t386, t1784
	CC1-MRSA-IV WA MRSA-1/57	New Zealand AK1 MRSA strain	ST1, ST1005, ST1115, ST1336	t127, t386, t590, t922, t2601
	CC1-MRSA-IV [PVL+] USA400	MW2, Canadian MRSA-7, New Zealand WR/AK1 MRSA	ST1	t127, t128, t175, t1784
	CC1-MRSA-IV+SCCfus WA MRSA-1/45	New Zealand AK1 MRSA strain	ST1	t127, t2279
	CC1-MRSA-IV+SCCfus [PVL+]	New Zealand WR/AK1 MRSA		
	CC1-MRSA-V			
	CC1-MRSA-V [PVL+]			t127
	CC1-MRSA-V+SCCfus			
	CC1-MRSA-V+SCCfus [PVL+]			
CC1 (ST567)	**ST567-MSSA [PVL+]**		ST567	t1242
CC1 (ST573/772)	**ST573/772-MSSA**			
	ST573/772-MSSA [PVL+]		ST772	t1839
	ST772-MRSA-V		ST772	t657
	ST573-MRSA-V WA MRSA-10		ST573	t5073
	ST772-MRSA-V [PVL+] Bengal Bay Clone60		ST772	t345, t657, t3387

MLST CC	Strain	Synonymes	MLST types associated with this strain	spa types associated with
CC1 (ST573/772)	**ST772-MRSA-V [PVL+] Bengal Bay Clone [ccr mutation/deletion]**			
CC5	**CC5-MSSA**		ST5, ST73	t002, t010, t053, t067, t088, t179, t214, t442, t548, t1062, t1265
	CC5-MSSA [PVL+]		ST5	t002, t311
	ST228-MRSA-I South German EMRSA/Italian Clone	Spanish PFGE type E6/9/15/17/18, UK-EMRSA 3	ST5, ST228	t001, t023, t041, t062, t110, t143, t149, t811, t892
	ST228-MRSA South German EMRSA (truncated SCCmec)		ST228	
	ST5-MRSA-I Geraldine Clone		ST5	t002
	CC5-MRSA-II [ACME+] WA MRSA-125			
	ST5-MRSA-II [tst1/mer+] Irish AR11	(USA100, Canadian MRSA-2)	ST5	t045
	ST5-MRSA-II [tst1+] New York-Japan Clone	USA100, Irish AR7.3/AR7.4, Canadian MRSA-2, N315, Mu50	ST5	t002, t045
	CC5-MRSA-II trunc. [kdp ccrA/B2 deletion]			
	ST5/ST225-MRSA-II Rhine-Hesse EMRSA/New York-Japan Clone	USA100, Canadian MRSA-2, Irish AR7.3/AR7.4, Finland E1, JH1/JH9	ST5, ST225, ST496	t002, t003, t014, t045, t067, t088, t102, t151, t242, t306, t548, t603, t627, t893, t1062, t1290, t2032, t2302, t2666, t3524, t7053
	CC5-MRSA-III	Belgium E3		t002
	CC5-MRSA-IV [ACME+]			
	CC5-MRSA-IV [fusC+] New Zealand AK3/WA MRSA-39		ST5,ST526	t002, t4065
	CC5-MRSA-IV [ORF CM14+] WA MRSA-122			
	CC5-MRSA-IV Paediatric clone	WA MRSA-03/25/50/71/74/82/105/111, USA800, Spanish PFGE type E7/8, Marseille CF clon	ST5, ST73, ST125, ST575, ST930, ST998	t002, t010, t067, t214, t306, t548, t640, t837, t6183, t7078
	CC5-MRSA-IV Paediatric clone [edinA+] WA MRSA-65		ST5, ST73	t002, t088
	CC5-MRSA-IV Paediatric clone [sed/j/r+]	USA800, Spanish PFGE type E7/8	ST5	t002, t045, t067, t088, t509, t548, t1818, t2173, t6183
	CC5-MRSA-IV Paediatric clone [tst1+]			
	CC5-MRSA-IV [PVL+/edinA+] WA MRSA-64/121	PVL-positive Paediatric clone	ST5	t311, t3778
	CC5-MRSA-IV [PVL+/fusC+/edinA+]	PVL-positive Paediatric clone	ST5	t1277
	CC5-MRSA-IV [PVL+/fusC+]	PVL-positive Paediatric clone	ST5	t311, t1277
	CC5-MRSA-IV [PVL+]	PVL-positive Paediatric clone	ST5	t002, t311, t450
	CC5-MRSA-IV+SCCfus			t447
	CC5-MRSA-IV+SCCfus Maltese Clone		ST149	t002, t105
	CC5-MRSA-IV+VI	Spanish PFGE type E7/8	ST73	t002, t067, t067, t2226, t6475

MLST CC	Strain	Synonymes	MLST types associated with this strain	spa types associated with
CC5	**CC5-MRSA-Ivar. WA MRSA-18/21/48/103**		ST5, ST835	t002, t570
	CC5-MRSA-SCC(MRSAZH47)/IV+V		ST641	
	CC5-MRSA-SCC(MRSAZH47)/IV+V [PVL+]		ST5	t311
	CC5-MRSA-V [ACME+] WA MRSA-80		ST5	t071
	CC5-MRSA-V [fusC+] WA MRSA-14	PVL-negative Variant of WA MRSA-109	ST5	t442
	CC5-MRSA-V [sec/d/j/l/r+] WA MRSA-87		ST835	t002
	CC5-MRSA-V [sed/j/r+] WA MRSA-11/34/35/90/108		ST5	t002, t045, t458, t688, t1265
	CC5-MRSA-V [tst+]			
	CC5-MRSA-V WA MRSA-81/85/86/123		ST5, ST1435	t002, t045, t242, t2666
	CC5-MRSA-V [PVL+]	PVL-positive Variant of WA MRSA-109		t002
	CC5-MRSA-VI New Paediatric Clone		ST5	t105, t777
	CC5-MRSA-VI New Paediatric Clone [PVL+]			
	ST5-MRSA-VII (SCC-JCSC6082)			
	CC5/ST835-MRSA-(NovelSCCmec) WA MRSA-40/46		ST835	t002
	CC5-MRSA with atypical SCCmec element			
	CC5-MRSA with atypical SCCmec element			
CC6	**CC6-MSSA**		ST6	t207, t701
	CC6-MSSA [PVL+]			
	CC6-MRSA-IV WA MRSA-51		ST6	
	CC6-MRSA-IV+V WA MRSA-66		ST6	t701
	CC6-MRSA-V			t5413
CC7	**CC7-MSSA**		ST7	t091
	CC7-MRSA-IV			
	CC7-MRSA-unknown SCCmec WA-MRSA-116		ST7	
	CC7-MRSA-V			t091
	CC7-MRSA-VI			
CC7 (ST1048)	**ST1048-MRSA-IV**		ST1048	t1081
CC8	**CC8-MSSA**		ST8, ST254, ST870	t008, t024, t190, t197, t211, t304, t334, t1029, t1854, t2169, t2953,t6021
	CC8-MSSA-SCCfus			t008, t024
	CC8-MSSA [ACME+]			t008
	CC8-MSSA [ccrA/B2+]			t024. t5694
	CC8-MSSA [PVL+]			t008, t400
	ST254-MRSA-(atypical SCCmec), Hannover EMRSA (subclone)		ST254	t009, t036

MLST CC	Strain	Synonymes	MLST types associated with this strain	spa types associated with
CC8	**ST247-MRSA-I, North German/Iberian EMRSA**	UK-EMRSA-5/8/17, Irish AR22, Irish New 02, Spanish PFGE type E1, Belgium E1, Finland E7/E10, France C	ST247	t051, t052, t194, t3503
	ST250-MRSA-I Early/Ancestral MRSA	Irish AR02, Irish Phenotype II	ST250, ST985	t008
	ST8-MRSA-IIA/B/D Irish AR13/14	Irish AR05, Irish-01, Irish New03	ST8	t190
	ST8-MRSA-IIA/B/D+SCC-M1 Irish AR13/14	Irish AR05, Irish-01, Irish New03	ST8, ST609	t064, t190, t2196
	ST8-MRSA-IIC/E Irish AR13/14	Irish AR05, Irish-01, Irish New03	ST8	t190
	ST8-MRSA-IIC/E+SCC-M1 Irish AR13/14	Irish AR05, Irish-01, Irish New03	ST8	t190
	ST8-MRSA-(IVF+ccrA/B-4)/-VI	Irish AR43/Irish-02 (Subclone with SCCmec IV F), ST8-MRSA-IV F, WA MRSA-16	ST8	t190
	ST8-MRSA-(IVG/E+ccrA/B-4) UK-EMRSA-12/13 Irish AR43	Irish-02, ST8-MRSA-IV G/E	ST8, ST94	t190, t4691
	CC8-MRSA-IV [sea+] Lyon Clone/UK-EMRSA-2	France A, France B	ST8, ST995, ST1337	t008, t024, t068, t121, t967, t2047, t2206, t4268
	CC8-MRSA-IV Lyon Clone (sea-neg. variant)/WA MRSA-88			
	CC8-MRSA-IV [sea-N315+]			
	CC8-MRSA-IV [sea+] UK-EMRSA-6			
	CC8-MRSA-IV [tst1+]	WA MRSA-104, tst1-positive Variants of WA MRSA-5/31	ST576	
	CC8-MRSA-IV UK-EMRSA-14/WA MRSA-5	ST8-MRSA IV, ST576-MRSA IV, ST1634-MRSA IV, WA MRSA-6/31/83	ST8, ST576, ST1634	t008, t334, t711, t1677
	CC8-MRSA-IV USA500	WA MRSA-20/58	ST008, ST507, ST609, ST612, ST1173	t064, t118, 451
	ST8-MRSA-IV [PVL+/ACME+] USA300	WA MRSA-12, Canadian MRSA-10, Spanish PFGE type A	ST8	t008, t024, t121, t211, t955, t4306
	ST8-MRSA-IV [PVL+/ACME-] ACME-negative variant of USA300	ACME-negative Variant of WA MRSA-12, Canadian MRSA-10, "Spanish/South American Variant of USA300"	ST8	t008
	ST8-MRSA-IV putative PVL-deletion mutant of USA300			
	CC8-MRSA-IV [PVL+ sed/j/k/q/r+] WA MRSA-62		ST923	t008, t1635, t9708
	CC8-MRSA-IV+ACME Danish spa t024/ST8-IV Strain		ST8	t024
	CC8-MRSA-IV+SCCfus			t008
	ST254-MRSA-IV+V UK-EMRSA-10/Hannover EMRSA		ST254	t024
	CC8-MRSA-IV+V WA MRSA-92		ST1757	t024
	CC8-MRSA-V	WA MRSA-53/120	ST8	t008
	CC8-MRSA-V WA MRSA-115		ST8	t008
	CC8-MRSA-V+SCCfus [ACME+] WA MRSA-77		ST8	t008
	CC8-MRSA-V+VI		ST983	t008

MLST CC	Strain	Synonymes	MLST types associated with this strain	spa types associated with
CC8	**CC8-MRSA-VI+SCCfus**	UK-EMRSA-12/13		t064
	CC8-MRSA-VIII WA MRSA-16		ST8	t024, t190
CC8 (ST72)	**ST72-MSSA**		ST72	
	ST72-MSSA-SCCfus			
	ST72-MSSA-SCCfus/kdp			
	ST72-MSSA [PVL+]			
	ST72-MRSA-IV USA700		ST72	t126, t324
	ST72-MRSA-IV [PVL+] WA MRSA-44		ST72	t791
	ST72-MRSA-V WA MRSA-91		ST72	t3092
	ST72-MRSA-V/SCCfus			
	ST72-MRSA-V+ccrA4r			
CC8 (ST239)	**ST239-MSSA**			
	ST239-MRSA-III Vienna/Hungarian/Brazilian Clone	Australian EMRSA-2 (AUS-2), New Zealand AKh4 MRSA, ATCC33592	ST239, (ST240, ST241)	t019, t030, t037, t363
	ST239-MRSA-III+SCCmer Vienna/Hungarian/Brazilian Clone	UK-EMRSA-4/7/9/11, Australian EMRSA-3 (AUS-3), Irish Phenotype III, Irish AR09/14/23/44, Canadian MRSA-3/6, New Zealand AKh4 MRSA, Finland E24, Greece 1	ST239, (ST240, ST241)	t030, t037, t074
	ST239-MRSA-[truncated SCCmec]			
	ST239-MRSA-III [ACME+]		ST239	t037
	ST239-MRSA-III+ccrA/B4			
	ST239-MRSA-IIIvar. [delta mecR negat.]+SCCmer	UK-EMRSA-1, Australian EMRSA-3 (AUS-3), Irish AR01 and AR15	ST239	t037
CC9	**CC9-MSSA**		ST9, ST903	t100, t209, t411
	CC9-MRSA-III			
	CC9-MRSA-IV		ST9	t1430
	CC9-MRSA-IV [PVL+] WA MRSA-126		ST1420	
	CC9-MRSA-IX			
	CC9-MRSA-V			
	CC9-MRSA-V-atyp/truncated			t899, t1234
	CC9-MRSA-atypical SCCmec/IXvar WA-MRSA 112		ST9	
CC9 (ST834)	**ST834-MSSA**			
	ST834-MRSA-IV WA MRSA-13	WA MRSA-33, WA MRSA-41	ST834, (formerly ST584, ST733)	t3029
	ST834-MRSA-IV [PVL+]			t724, t1379
	ST834-MRSA-VI/SCCfus?			
CC10	**CC10-MSSA**		ST10	t166, t864
CC12	**CC12-MSSA**		ST12, ST901	t156, t160, t771, t888, t4490
	CC12-MRSA WA MRSA-59		ST12	t160
	CC12-MRSA-IV WA MRSA-69		ST12	t160

MLST CC	Strain	Synonymes	MLST types associated with this strain	spa types associated with
CC15	**CC15-MSSA**		ST15, ST582, ST869, ST1025	t084, t085, t094, t254, t360, t774, t1509, t1885
	CC15-MSSA [PVL+]		ST15	t360
	CC15-MRSA-I			
CC15 (ST199)	**ST199-MSSA**		ST199	t774
CC20	**CC20-MSSA [lukF-P83/M+]**			
	CC20-MSSA		ST20, ST389	t148, t195, t1023
	CC20-MRSA-V			
CC22	**CC22-MSSA**		ST22	t005, t395, t3242
	CC22-MSSA [PVL+]			t005, t310, t417, t891
	CC22-MSSA-SCCfus [PVL+]			t417, t6101
	CC22-MRSA-IV [ACME+] UK-EMRSA-15/Dublin variant		ST22	t022, t032, t3185
	CC22-MRSA-IV [fnbB-,sec/l-] UK-EMRSA-15/Barnim EMRSA	Irish AR06, Canadian MRSA-8, Spanish PFGE type E13, "ST22-A" clade of UK-ERMSA-15	ST22, ST1117	t020,t022,t025,t032,t432,t515,t578,t717,t790,t1214,t1559,t1802,t2951
	CC22-MRSA-IV [fnbB-,sec/l+] UK-EMRSA-15/Barnim EMRSA	Irish AR06, Canadian MRSA-8, Spanish PFGE type E13, "ST22-A" clade of UK-ERMSA-15	ST22	t020,t022,t032,t432,t531,t981,t1214,t1370,t1802,t1865,t2235,t3213,t3501,t3505
	CC22-MRSA-IV [fnbB+] UK-EMRSA-15/Barnim EMRSA	Irish AR06, Canadian MRSA-8, Spanish PFGE type E13, "ST22-non A" clade of UK-ERMSA-15	ST22	t005, t032, t451, t2951
	CC22-MRSA-IV [fusC+] UK-EMRSA-15/Maltese variant			
	CC22-MRSA-IV [tst1+] UK-EMRSA-15/Middle Eastern variant		ST22	t032, t223, t309, t1977, t5711
	CC22-MRSA-IV [PVL+]		ST22	t005, t016, t310, t852, t2518, t2647
	CC22-MRSA-IV [ACME+/PVL+]		ST22	t2480
	CC22-MRSA-V			
	CC22-MRSA-V [PVL+]			
CC25	**CC25-MSSA**		ST25, ST28, ST1017	t078, t140, t287, t349, t660, t1521, t6145
	CC25-MSSA [PVL+]		ST25, ST28	t078, t1054, t2554
CC30	**CC30-MSSA [lukF-P83/lukM+]**			
	CC30-MSSA		ST30, ST39	t012, t017, t018, t021, t122, t338, t363, t797
	CC30-MSSA [PVL+]	Phage type 80/81, V8 strain, Cowan I, ATCC12598, ATCC25923, ATCC49775, Oxford Staph.	ST30	t019, t021, t076, t300, t318, t582, t8687
	ST36/39-MRSA-II UK-EMRSA-16	USA200, Irish AR7.0, Canadian MRSA-4, Spanish PFGE type E12, Finland E5	ST36, ST39	t007, t012, t018, t253, t419, t924
	CC30-MRSA-IV [PVL-/tst1-]			
	CC30-MRSA-IV [PVL-/tst1+] WA MRSA-68		ST39	t018, t2643
	CC30-MRSA-IV [PVL+] Southwest Pacific Clone	USA 1100, West Samoan Phage Pattern (WSPP) Clone	ST30, ST36	t019, t021, t122, t300, t318, t975, t5447, t7561
	CC30-MRSA-IV+SCCfus		ST30	t012, t021
	CC30-MRSA-IV+SCCfus [PVL+]			
	CC30-MRSA-IV+VI WA MRSA-102			

MLST CC	Strain	Synonymes	MLST types associated with this strain	spa types associated with
CC30	**CC30-MRSA-V**			
	CC30-MRSA-V [PVL+] WA MRSA-124			
CC30 (ST34/42)	**ST34/42-MSSA**		ST34, ST42	t089, t136, t166, t582, t2096
CC45 (agr I)	**CC45-MSSA**		ST45, ST508, ST1008, ST1009	t015, t026, t050, t065, t116, t133, t339, t362, t383, t390, t397, t465, t576, t1331, t1608, t2714
	CC45-MSSA [egc deletion variants]		ST45	t065, t330
	CC45-MSSA [PVL+]		ST45	
	CC45-MRSA-II USA600	USA600-MRSA-IV, WA MRSA-75, Belgium E2	ST45	t266
	CC45-MRSA-IV [ACME+]			
	CC45-MRSA-IV [tst1+/ACME+]			
	CC45-MRSA-IV [tst1+]			t2674
	CC45-MRSA-IV Berlin EMRSA	USA600-MRSA-IV, WA MRSA-75, Belgium E2	ST45	t004, t007, t015, t040, t302, t750, t950, t1424, t2135
	CC45-MRSA-IV+SCCfus			
	CC45-MRSA-V			t015, t1156
	CC45-MRSA-V [ACME+] WA MRSA-106			
	CC45-MRSA-V [tst1+] WA MRSA-4		ST45	t123
	CC45-MRSA-V [PVL+]			
	CC45-MRSA-V+VI			
CC45 (agr IV)	**CC45/agrIV-MSSA**			
	CC45/agrIV-MSSA [capsule type 5]		ST45	t6552
	CC45/agrIV-MRSA-IV WA MRSA-23		ST45	t727, t1575
	CC45/agrIV-MRSA-IV+V			
	CC45/agrIV-MRSA-VT WA MRSA-84		ST45	t1081
CC45 (ST617)	**ST617-MRSA-IV**		ST617	t305
CC49	**CC49-MSSA [lukF-P83/lukM+]**		ST49	t208
	CC49-MSSA		ST49	t208
	CC49-MSSA [PVL+]		ST49	
	CC49-MRSA-V		ST49	
CC50	**CC50-MSSA**		ST50	t246
	CC50-MRSA-V+SCCfus			
CC59	**CC59-MSSA**		ST59, ST375	t216, t1151, t3736
	CC59-MSSA [PVL+]		ST59	t437
	ST59-MRSA-IV WA MRSA-118	PVL-negative Variant of WA MRSA-55/56	ST59	t437
	ST59-MRSA-IV WA MRSA-73		ST59	t172, t528
	ST87-MRSA-IV WA MRSA-24		ST87	t216
CC59	**CC59-MRSA-IV [PVL+] USA1000**		ST59	t216, t316

MLST CC	Strain	Synonymes	MLST types associated with this strain	spa types associated with
CC59	**ST59-MRSA-IV [PVL+] WA MRSA-55/56**		ST59	t437
	ST59-MRSA-IV+V WA MRSA-15		ST59	t976
	ST59-MRSA-V		ST59	t316, t441
	CC59-MRSA-V [PVL+]		ST59	t316
	ST59/952-MRSA-V(T) [PVL+] Taiwan Clone	WA MRSA-9, WA MRSA 52	ST59, ST952	t437, t441, t1950, t2365
	CC59-MRSA-V+SCCfus			
CC80	**CC80-MSSA**			
	CC80-MSSA [PVL+]		ST80	t044
	atypical CC80-MSSA [ORF CM14/PVL+]		ST80 slv	t1849
	CC80-MRSA- (truncated/atypical SCCmec)			
	CC80-MRSA- (truncated/atypical SCCmec) [PVL+]			
	CC80-MRSA-IV			
	CC80-MRSA-IV [PVL+] European caMRSA Clone	WA MRSA-17/30	ST80, ST583, ST728	t042, t044, t131, t203, t416, t434
CC88	**CC88-MSSA**		ST78, ST88	t186, t729, t730
	CC88-MSSA [PVL+]		ST88	t693, t729, t1598, t4195
	CC88-MRSA-IV WA MRSA-2		ST78, ST257	t186, t690, t3205
	CC88-MRSA-IV [etA+]		ST88	t186, t690, t786
	CC88-MRSA-IV [PVL+]		ST88	t690, t692
	CC88-MRSA-V			t186
	CC88-MRSA-V [PVL+] WA MRSA-117			
	CC88-MRSA-Vtrunc. [PVL+]			t1764
	CC88-MRSA-VI			
	CC88-MRSA-VII (SCC-JCSC6082)		ST129	
ST93	**ST93-MSSA**			
	ST93-MSSA [PVL+]			t202, t4178, t4699, t5767, t6485
	ST93-MRSA-IV [PVL-]			t1811, t6847
	ST93-MRSA-IV [PVL+] Queensland Clone	WA MRSA-7		t202, t4178
	ST93-MRSA-V			t202
	ST93-MRSA-V [PVL+]			
CC96	**CC96-MSSA**			
	CC96-MSSA [PVL+]		ST96	
	ST154-MRSA-IV [PVL+] Central Asian caMRSA/WA MRSA-119		ST154, ST1930	t667
CC97	**CC97-MSSA [lukF-P83/lukM+]**		ST97, ST352	
	CC97-MSSA	ATCC6538,	ST97, ST115, ST464	t044, t131, t224, t267, t359, t521,t524, t528, t1234
	CC97-MSSA [ccrA/B2+]			

MLST CC	Strain	Synonymes	MLST types associated with this strain	spa types associated with
CC97	**CC97-MSSA-SCCmer**	Wood46	ST97	t359
	CC97-MRSA-(I+V)			
	CC97-MRSA-IV WA MRSA-54/63		ST953, ST1174	t267, t1359
	CC97-MRSA-V		ST97	t1234
	CC97-MRSA-V+ACME			
CC97 (ST71)	**ST71-MSSA**		ST71	t524
CC101	**CC101-MSSA**		ST101	t056, t150, t1312
CC121	**CC121-MSSA**		ST121	t159, t272, t850, t2155
	CC121-MSSA [PVL+]		ST121	t159, t284, t314, t435, t518, t645, t4197
	CC121-MRSA-IV			
	CC121-MRSA-V WA MRSA-22		ST577	t3025
	CC121-MRSA-V [PVL+]			
	CC121-MRSA-VT WA MRSA-93		ST121	t159
CC126	**CC126-MSSA [bap-]**			
	CC126-MSSA [bap+]			
CC130	**CC130-MSSA [lukF-P83/lukM+]**		ST700, ST2011, ST2024	t524, t3568, t8403
	CC130-MRSA-XI		ST130, ST1245, ST1764	t843, t6220
CC133	**CC133-MSSA**		ST133, ST2111	t1166, t1181, t4648, t6384
	CC133-MSSA [capsule type 5]		ST2111	t2379
	CC133-MSSA [lukP83/M+]		ST132, ST133, ST1452	t1403, t2678
CC136	**ST136-MSSA**		ST136	
ST140	**ST140-MSSA**			
	ST140-MRSA-IV			t957
CC152	**CC152-MSSA**			
	CC152-MSSA [PVL+]		ST152	t355, t4690
	CC152-MRSA-V			
	CC152-MRSA-V [PVL+]	WA MRSA-89	ST152, ST377, ST1633	t355, t1096
CC182	**CC182-MSSA**		ST182	t364
	CC182-MSSA [ccrA/B-2/kdp+]		ST182, ST944	t364, t493, t1406, t1772
CC188	**CC188-MSSA**		ST188	t189,t3380
	CC188-MSSA [PVL+]			
	CC188-MRSA-IV WA MRSA-38/78		ST188	t189
	CC188-MRSA-V			
CC188 (ST1774)	**ST1774-MRSA-IV+ACME**		ST1774	t1081
ST350	**ST350-MSSA**		ST350	t1106
CC361	**CC361-MSSA**		ST361	
	CC361-MRSA-IV WA MRSA-29		ST361, ST672	t315, t1309
	CC361-MRSA-V WA MRSA-70		ST672	t1309
	CC361-MRSA-V			

MLST CC	Strain	Synonymes	MLST types associated with this strain	spa types associated with
	WA MRSA-110			
CC361	**CC361-MRSA-VIII** **WA MRSA-28**		ST361	t315
CC395	**CC395-MSSA**		ST395, ST426, ST1012	t271, t412, t536, t900
	CC395-MRSA-IV			
CC398	**CC398-MSSA**		ST398	t034
	CC398-MSSA [PVL+]			t034
	CC398-MRSA-(truncated SCCmec V or X)			t034, t3081
	CC398-MRSA-IV		ST398	t011, t899
	CC398-MRSA-V	Dutch livestock-associated MRSA, "Dutch Pig Strain"	ST398	t011, t034, t108, t571, t1197, t1250, t1451, t1456, t1606, t2346, t2510
	CC398-MRSA-V [PVL+]			t034
	CC398-MRSA-X			
CC398 (ST291/813)	**ST291/813-MSSA**		ST291, ST813	t1149
	ST291/813-MSSA [PVL+]		ST291	t1149
ST425	**ST425-MSSA**			t6386
	ST425-MRSA-XI			t6292
CC479	**CC479-MSSA**			
	CC479-MSSA [lukF-P83/lukM+]		ST479, ST1275	t2873, t7013
CC509	**CC509-MSSA**		ST509	t375
	CC509/ST207-MRSA-V		ST207	
CC522	**CC522-MSSA [lukF-P83/lukM+]**		ST522	
CC599	**CC599-MRSA-XI**		ST599	t5930
	CC599-MSSA			
CC692	**CC692-MSSA**		ST692	
CC705	**CC705-MSSA [lukF-P83/lukM+]**	CC151-MSSA, RF122	ST151, ST504, ST705, ST1274	t528, t529
CC707	**CC707-MSSA**		ST707	t1458, t3630
	CC707-MSSA [ccrA/B-2/kdp+]		ST707	
CC779	**CC779-MSSA**			
	CC779-MSSA-SCCfus			t878
	CC779-MRSA-IV **WA MRSA-100**			
	CC779-MRSA-V+SCCfus		ST779	t878
	CC779-MRSA-(novel SCCmec)+SCCfus		ST779	t878
ST816	**ST816-MSSA**			t1294
ST890	**ST890-MSSA**			t1773
ST913	**CC913-MRSA-IV**			t991
CC942	**CC942-MSSA**		ST942	t1445
	CC942-MSSA [PVL+]			
CC1021	**CC1021-MSSA**		ST918, ST1021	
	CC1021-MSSA [PVL+]			

MLST CC	Strain	Synonymes	MLST types associated with this strain	spa types associated with
ST1093	**ST1093-MSSA**			
ST1290/2481	**CC1290-MSSA**			t131
ST1643	**ST1643-MSSA**			t6385
ST1755	**ST1755-MSSA**			
ST1852	**ST1852-MSSA**			
CC1943	**CC1943-MSSA**			t978
	CC1943-MRSA-XI	ST2361-MRSA-XI	ST2361	t978
ST2249	**ST2249-MRSA-III**			t037
ST2279	**ST2279-MSSA**			
ST2425	**ST2425-MSSA**			
ST2479	**ST2479-MSSA**			
	ST2479-MSSA [PVL+]			
ST2482	**ST2482-MSSA [PVL+]**			
ST2691	**ST2691-MSSA**			
S. argenteus lineage: CC75	**CC75-MSSA**		ST75	
S. argenteus lineage: CC75	**ST75-MRSA-IV WA MRSA-8/79**		ST75	t(259-31-17-17-17-17-23-17-17-23-17-22) and related
S. argenteus lineage: CC75	**ST75-MRSA-V**			
S. argenteus lineage: ST883	**ST883-MRSA-IV WA MRSA-47**			t(259-23-23-17-17-17-23-23-23-17-16)
S. argenteus lineage: CC1223/1594	**CC1223/1594-MSSA**		ST1223, ST1594, ST1719	
S. argenteus lineage: ST1303	**ST1303-MRSA-IV WA MRSA-76**			t(259-25-17-17-16-16-16-16-16)
S. argenteus lineage: CC75	**ST1304-MRSA-IV WA MRSA-72**			t(259-31-17-17-17-17-23-17-17-23-17-22)
S. argenteus lineage: ST2250/2277	**ST2250/2277-MSSA**		ST2250, ST2277 and related	
S. argenteus lineage: ST2250/2277	**ST2250-MRSA-IV WA MRSA-114**		ST2250 and related	t6675
S. argenteus lineage: ST2250/2277	**ST2250/2277-MRSA-V WA MRSA-113**			